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STUDIES ON PROBABLE DAMAGE BY BLISTER RUST IN SOME REPRESENTATIVE STANDS OF YOUNG WESTERN WHITE PINE [1]

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INTRODUCTION

It has long been known that white-pine blister rust (*Cronartium ribicola* Fisch.) is able to kill pines of susceptible species. Quantitative studies of damage by this disease are obviously essential to the formulation of satisfactory management and disease-control policies for white pine stands. Snell (*7, 8, 9, 10*) [3] and others have discussed the effect of the rust on young eastern white pines (*Pinus strobus* L.). Lachmund (*5*) has described examples of damage to commercially immature western white pines (*P. monticola* Dougl.), and Buchanan (*1*) has recently demonstrated the destructiveness of the disease to merchantable trees of the same species.

This paper is concerned with the relationship between intensity of infection and degree of damage, and with some of the more important factors affecting this relationship, in stands of western white pine less than 50 feet in height. Even in very small trees the killing of occasional branches is of little or no material importance. When conditions for pine infection are very favorable, cankers may sometimes be so numerous as to cause the death of trees simply by killing most of the branches individually (i. e., without girdling the trunks) (*5*), but in such cases the amount of infection present far exceeds the minimum necessary to cause the same damage by trunk girdling within a few additional years. Throughout this paper, therefore, only those cankers that have entered or may be expected to enter the trunks are considered to be injurious to the host.

METHODS

The studies were generally confined to areas where practically all of the cankers had originated in a single year or in two consecutive years as indicated by Lachmund's method (*3*) of determining the age of infection on western white pine, and data were usually taken only on those cankers that appeared to have originated during the year or years of heaviest infection. In most cases the trees were examined as soon as the cankers were large enough to be found easily (about 5 years after the occurrence of the infection). The following data were

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[3] Italic numbers in parentheses refer to Literature Cited, p. 568.

recorded for every infected tree: Height at time of infection and at time of examination, length and width of crown at time of infection (except at Brackendale, where crown dimensions were taken as of the year of examination), number of cankers, and number of cankers capable of causing damage. In British Columbia the following additional data were usually taken on each canker: Distance from ground, distance from trunk, and probable date when damage would be caused. Where not otherwise specified, all data presented are as of the year in which infection occurred.

Lachmund (*4, pp. 491–499*) has published a detailed description of the principal factors that determine whether or not a given canker will ever enter the trunk, and of the method of calculating the time and severity of the injury that will be caused by cankers able to enter. The studies reported in this paper are based almost entirely upon such calculations, since few stands of western white pine have as yet been exposed to the rust long enough for damage from even the older cankers to manifest itself fully, and since successive waves of infection are usually so heavy as to prevent any determination of the damage that might result from smaller numbers of cankers. For the same reasons it has been impossible to make extensive comparisons of calculated results with actual results; however, a careful reexamination (about 5 years after the original examination) of 959 cankers on one of the study areas (Revelstoke) indicated that damage, although somewhat slower than had been expected, would conform very closely to the original calculations.

The potential effect of each individual canker was determined without reference to any other canker. For example, a canker that would kill its host in 20 years if it were the only canker present was recorded as a fatal infection even if another canker was present that would kill in 15 years. Cankers were classified in three groups: (1) Those that would kill (this group included a majority of the cankers capable of damaging, even in trees from 40 to 50 feet tall), (2) those that would seriously injure (*4, p. 498*), and (3) those that would never enter the trunk. It makes little difference from a practical standpoint, however, whether a tree is killed or only injured to such an extent that its potential value is largely destroyed; accordingly, throughout this paper no distinction is made between cankers capable of killing and those capable only of causing serious injury.

STUDY AREAS

The studies were initiated in 1928 on an area of level land at a low elevation near Brackendale in the coastal region of British Columbia. Very few ribes were present on this study area, but large numbers of some of the most susceptible species, including *Ribes bracteosum* Dougl., *R. divaricatum* Dougl., and a few plantings of *R. nigrum* L., occurred around it. Western white pines ranging from 10 to 60 feet in height were mixed with Douglas fir (*Pseudotsuga taxifolia* (Lam.) Britt.) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) in a stand of rather uneven density. Blister rust became established in this locality about 1913. Moderately heavy pine infection occurred during 1917 and 1918, and again during 1920 and 1921. Only those cankers originating during 1917 and 1918 were included in the study. Since practically all of the sporidia came from ribes at some distance from the study area, pine infection was distributed fairly uniformly throughout the stand.

The studies were continued during 1929 to 1932, inclusive, near Revelstoke,[4] in eastern British Columbia about 135 miles north of the international boundary. This area was situated on a steep slope just above the Columbia River, and ranged from about 1,800 to about 2,400 feet in elevation. *Ribes lacustre* (Pers.) Poir. occurred commonly along a small stream, and scattered bushes of the same species were present in various other parts of the area. The nearest ribes of any other species were several miles away. Western white pines, mostly less than 25 feet tall, were mixed with other conifers, willows, aspen, alders, etc., in a stand of rather irregular density. The rust probably became established here about 1917. A light wave of pine infection occurred in 1922, a few additional cankers originated in 1924 and 1925, and several thousand cankers originated during 1927 and 1928. Data were taken on all cankers, regardless of their age. The cankers on this area have been separated into two groups: Those originating in 1922, and those originating during 1924 to 1928, inclusive. All cankers of the latter group are herein assumed, for the sake of convenience, to have originated in 1927 (the year in which more than 80 percent of the total infection occurred).

An area [5] at an elevation of approximately 2,000 feet near Hunter's Siding (about 50 miles south of Revelstoke) was examined during 1930 to 1933, inclusive. Western white pines, averaging 443 per acre, were mixed with other conifers and various hardwoods in a fairly dense stand. Infection here originated in 1928 and in 1930 from inoculated ribes at the center of the area. Data were taken on all cankers. Tree dimensions are herein given as of 1928 (the year in which slightly more than 50 percent of the infection occurred).

By 1933 the disease had become common and conspicuous on pines at several infection centers in Idaho. The following five areas in that State were examined during that year: Crystal Creek (east of Fernwood, Benewah County), St. Maries River (east of Clarkia, Shoshone County), Gold Center Creek (east of Clarkia), Deep Creek (south of Elk River, Clearwater County), and Elk River (south of Elk River). These areas were situated on gentle to moderate slopes at elevations ranging from about 2,000 to about 3,500 feet. White pine was the principal tree species on all the areas except the one at Deep Creek, where Douglas fir and western hemlock were also abundant. The stands were moderately dense at Crystal Creek and St. Maries River, somewhat less so at Deep Creek, irregular but generally open at Elk River, and quite open at Gold Center Creek. In every case, highly susceptible stream-type ribes had been abundant in the immediate vicinity of the pines, but these bushes had recently been destroyed except at Crystal Creek. Practically all of the cankers, and all of those included in the study, were of 1927 origin at Crystal Creek and St. Maries River, of 1928 origin at Deep Creek and Elk River, and of 1930 origin at Gold Center Creek.

On the Revelstoke, Hunter's Siding, and Gold Center Creek areas, detailed data were taken on all trees, both infected and uninfected. On the rest of the Idaho areas, and at Brackendale, no record was kept of uninfected trees, and infected trees were ignored whenever a

[4] A more detailed description of this area is given by Mielke (*6*). The tree and canker bases used in this paper differ slightly from those used by Mielke, since insufficient data on some of the cankers necessitated their omission and since a few cankers that may have originated in 1929 have been included.

[5] Described in more detail by Buchanan and Kimmey (*2*).

TABLE 1.—*Number of cankers and infected trees, by tree-height classes, used on the various study areas*

Tree-height class (feet)	Brackendale		Revelstoke [1]		Revelstoke [2]		Hunter's Siding		Crystal Creek		St. Maries River		Gold Center Creek		Deep Creek		Elk River		Total	
	Infected trees	Cankers	Infected trees	Cankers	Infected trees	Cankers	Infected trees	Cankers	Infected trees	Cankers	Infected trees	Cankers	Infected trees	Cankers	Infected trees	Cankers	Infected trees	Cankers	Infected trees	Cankers
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
0.1–5.0	0	-------	21	21	206	239	11	20	30	118	30	171	119	178	68	83	31	51	516	881
5.1–10.0	13	39	61	64	469	739	30	42	32	370	32	189	69	189	73	118	52	169	831	1,919
10.1–15.0	51	242	93	98	509	1,025	41	85	31	216	30	175	8	19	25	50	45	210	833	2,120
15.1–20.0	51	177	113	124	598	1,628	21	60	30	191	29	268	0	-------	8	23	38	312	888	2,783
20.1–25.0	50	315	18	18	786	2,603	14	19	33	336	31	305	0	-------	2	29	33	268	967	3,893
25.1–30.0	51	377	0	-------	546	2,215	2	2	30	252	31	277	0	-------	0	-------	32	410	692	3,533
30.1–35.0	50	433	0	-------	201	1,098	0	-------	30	192	30	354	0	-------	1	11	31	402	343	2,490
35.1–40.0	50	467	0	-------	33	213	0	-------	30	329	30	229	0	-------	0	-------	37	369	180	1,607
40.1–45.0	28	255	0	-------	3	15	0	-------	31	332	31	322	0	-------	0	-------	25	173	118	1,097
45.1–50.0	10	112	0	-------	0	-------	0	-------	30	513	27	201	0	-------	0	-------	19	154	86	980
Total	354	2,417	306	325	3,351	9,775	119	228	307	2,849	301	2,491	196	386	177	314	343	2,518	5,454	21,303

[1] Infection originating in 1922.

[2] Infection originating in 1924 to 1928, inclusive.

sufficient representation of the height class to which they belonged had already been secured on that area.

All of the areas, in both British Columbia and Idaho, were representative of good white pine sites in their respective regions. Practically all of the trees included in these studies were dominant or codominant. The number of cankers and the number of infected trees on each of the study areas are given by tree-height classes in table 1.

RELATIONSHIP BETWEEN INFECTION AND DAMAGE IN INDIVIDUAL TREES

Infections occasionally occur on the needle-bearing portion of the main stem and on the small secondary branches common in the interior of western white pine crowns, but most of the foliage, and hence most of the infections, occur near the ends of the primary branches and their principal ramifications. In small trees (i. e., up to about 5 feet in height) the branches are so short that a majority of the cankers are able to grow into the main stem, where their action is almost invariably fatal because of the extensive development of the crown above the infected whorl during the period between infection and stem girdling, and because cankers on stems of young trees are usually able to continue downward growth until the base of the crown is reached. In larger trees, cankers may be grouped in three intergrading classes, in decreasing order of frequency, as follows: (1) Those originating in the lower part of the crown, which are generally unable to enter the trunk because they are usually situated near the ends of long branches; (2) those originating in the intermediate part of the crown, which are frequently able to enter the trunk and in such cases are almost always fatal; (3) those originating in the upper part of the crown, which are usually able to enter the trunk but which may merely injure rather than kill if a large part of the crown still exists below the infected whorl when the trunk is girdled and if the canker is subsequently unable to grow down to the base of the crown.

Most cankers occur in the lower portions of the crowns, principally because much greater quantities of foliage are present there than in the tops of the trees. It might therefore be expected that the percentage of cankers capable of causing damage would be much less in larger trees, whose lower branches are too long to be traversed by very many of the cankers originating near their tips, than in smaller trees. Figures 1 and 2 show this to be the case. Many of the differences apparent between the various curves in these two figures were also at least partially attributable to differences in crown width. For example, crown widths (by 5-foot tree-height classes) for trees from 15.1 to 50.0 feet tall ranged from 6 to 10 feet at Crystal Creek and from 8 to 16 feet at St. Maries River, while percentages of cankers capable of causing damage in these height classes (fig. 2) ranged from 21 to 32 and 15 to 23, respectively. These areas appeared fairly similar in other respects, and it seems highly probable that crown width was the factor principally responsible for the difference between these two percentage ranges. On the other hand, the percentage of cankers capable of causing damage to trees of any given height class on the Revelstoke area (fig. 1) was distinctly greater in the infection wave of 1922 than in that of 1927, although average crown widths by height classes in the latter year did not differ appreciably from those in the former. In

1922 this stand was relatively open, and in only a few cases was suppression of infected branches likely to prevent growth of cankers into the trunks. By 1927 the coniferous and hardwood cover was begin-

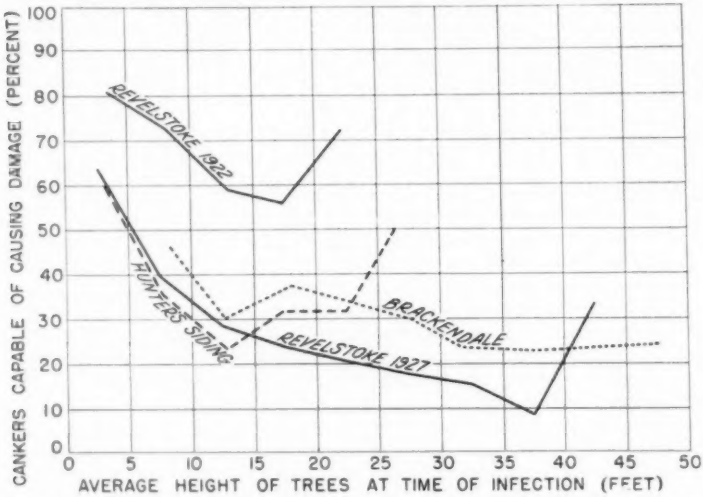

FIGURE 1.—Percentage of cankers capable of causing damage in the various height classes on study areas in British Columbia.

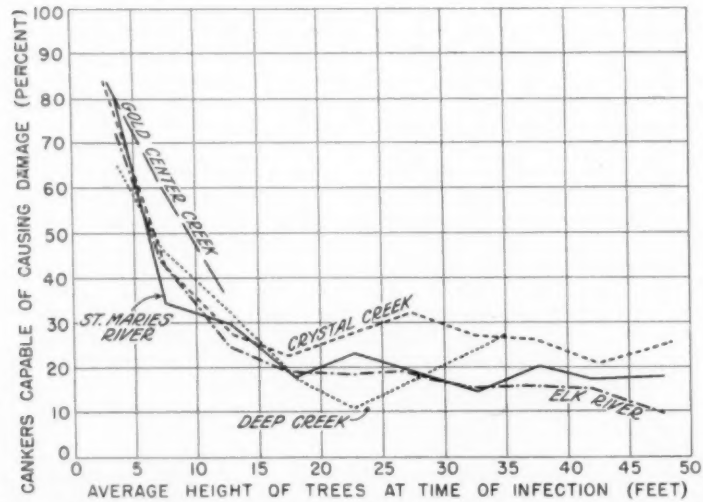

FIGURE 2.—Percentage of cankers capable of causing damage in the various height classes on study areas in Idaho.

ning to close over much of the area, and it was obvious that cankers of 1927 origin at increasing distances from the trunks were progressively less likely to enter than were those of 1922 origin (table 2).

TABLE 2.—*Percentages of cankers, originating at different distances from the trunks on the Revelstoke area, capable of causing damage* [1]

Distance of canker origin from trunk (feet)	Canker basis [2]		Cankers capable of causing damage		Distance of canker origin from trunk (feet)	Canker basis [2]		Cankers capable of causing damage	
	1922 infection	1927 infection	1922 infection	1927 infection		1922 infection	1927 infection	1922 infection	1927 infection
	Number	*Number*	*Percent*	*Percent*		*Number*	*Number*	*Percent*	*Percent*
0.1–1.0	59	536	93	87	5.1–6.0	6	468	0	[3] +
1.1–2.0	116	1,418	81	49	6.1–7.0	0	121	----	2
2.1–3.0	85	1,786	44	16	7.1–8.0	0	28	----	0
3.1–4.0	37	1,448	22	3	8.1–9.0	0	7	----	0
4.1–5.0	14	1,054	7	[3] +					

[1] Data from all tree-height classes.
[2] Total basis differs from that given in table 1 because data on distance from trunk were not available for all cankers.
[3] The plus signs signify percentages greater than 0 but less than 0.5.

Unfortunately, the available data are insufficient to illustrate the relation between crown length and severity of damage. Other things being equal, it is evident that shortening of the crowns will not result in a proportional reduction in the number of cankers capable of damaging, since such cankers usually originate in the upper portions of the crowns. In reality, however, trees subjected to lateral suppression differ in many respects from those receiving full side light, and the probable extent of damage from a given degree of infection in a closed stand consequently cannot be even approximately predicted from studies of more open stands, or vice versa.

Table 3 shows that the period between infection and actual occurrence of damage is longer, on an average, in large than in small trees. The most rapid increase in the length of this period apparently takes place in trees less than about 10 feet tall. Both crown widths and distances of cankers in general from the trunks are considerably greater in larger trees, but cankers near the ends of long branches are rarely able to cause damage and the average distance of potentially damaging cankers from the trunks of such trees is accordingly only slightly greater than in trees 10 feet tall. Then, too, cankers grow more rapidly on thick branches than on slender ones (*4*), and on long branches may consequently grow at rates that partially compensate for the greater distances to be traversed.

Lachmund (*4*) found canker growth rates to be somewhat higher on branches of the same diameter at low elevations in the coastal region of British Columbia than at Revelstoke. The average time required for damage to occur in a given height class might therefore be expected to be somewhat shorter at Brackendale than at Revelstoke, since table 3 shows that average distances from trunks to points of origin of potentially damaging cankers were approximately the same on these two areas. At Brankendale, however, the branches were unusually slender and growth of cankers was correspondingly slow.

From the foregoing discussion it will be seen that numerous factors may cause appreciable variation in the relationship between intensity of infection and probability of damage by blister rust, even in adjacent trees. The data presented herein indicate, however, that consider-

able damage can be caused by relatively light infection in trees less than 50 feet tall, and that such damage will generally occur before commercial maturity under western standards is attained.

TABLE 3.—*Distance from trunk to point of origin of potentially damaging cankers, and average time required for damage to occur*

Tree height class (feet)	Revelstoke [1]		Brackendale		Tree height class (feet)	Revelstoke [1]		Brackendale	
	Average distance from trunk to origin of potentially damaging cankers	Average time from infection to damage	Average distance from trunk to origin of potentially damaging cankers	Average time from infection to damage		Average distance from trunk to origin of potentially damaging cankers	Average time from infection to damage	Average distance from trunk to origin of potentially damaging cankers	Average time from infection to damage
	Feet	*Years*	*Feet*	*Years*		*Feet*	*Years*	*Feet*	*Years*
0.1–5.0	0.5	11			25.1–30.0			2.0	20
5.1–10.0	1.2	16	1.1	17	30.1–35.0			1.9	20
10.1–15.0	1.7	18	1.8	18	35.1–40.0			2.1	21
15.1–20.0	2.0	20	1.6	19	40.1–45.0			2.4	23
20.1–25.0	2.1	20	1.9	19	45.1–50.0			2.1	22

[1] Infection originating in 1922.

The effect of blister rust on trees more than about 50 feet in height cannot be even approximately predicted by extrapolation of the data herein presented. Crowns change in form and character as the rate of height growth diminishes, and these changes become most pronounced in trees between 50 and 100 feet tall. Since the structure of the crowns very largely determines the extent of the damage likely to result from a given number of cankers, it is evident that conclusions as to the probable liability of infected mature and nearly mature trees to damage can be derived only from studies of such trees.

EFFECT OF TREE SIZE ON LIABILITY TO INFECTION AND RESULTANT DAMAGE

Large trees usually have much more foliage and, as a result, are much more subject to infection than are smaller trees exposed to the same conditions. Consequently, although the percentage of cankers capable of causing damage decreases with increasing tree size (figs. 1 and 2), percentages of total trees damaged are frequently, if not always, greater in the larger size classes (up to at least 40 feet in height) of uneven-aged stands of reproduction. This is shown in table 4, where infection and damage percentages in the various height classes are given for the Revelstoke, Hunter's Siding, and Gold Center Creek areas.

This relationship between size of tree and liability to damage appears fundamentally unlike that described by Snell in *Pinus strobus* (*7*, *8*). Snell's results cannot be directly compared with those of the present study, because he considered as damaged only those trees that would be killed by the rust at ages of less than 50 years, and because his estimates of damage in the various height classes are expressed as percentages of infected trees instead of as percentages of

total trees; his data and discussion, however, indicate that infection of *P. strobus* is most severe among the smallest trees. The dissimilarity between the two species must therefore be partially attributable to differences in conditions governing distribution of infection, and possibly also to differences in development of and host reaction to established cankers, as well as to the use of different criteria in the estimation of damage.

TABLE 4.—*Infection and damage, by tree-height classes, on the Revelstoke,*[1] *Hunter's Siding, and Gold Center Creek areas*

Area and tree-height class (feet)	Basis		Average per tree		Trees infected	Trees damaged
	Trees	Cankers	Total cankers	Cankers capable of damaging		
Revelstoke:	*Number*	*Number*	*Number*	*Number*	*Percent*	*Percent*
0.1–5.0	3,107	239	0.1	[2]+	7	5
5.1–10.0	2,046	739	.4	0.1	23	12
10.1–15.0	1,218	1,025	.8	.2	42	19
15.1–20.0	1,160	1,628	1.4	.3	52	23
20.1–25.0	1,258	2,603	2.1	.4	62	27
25.1–30.0	812	2,215	2.7	.5	67	29
30.1–35.0	274	1,098	4.0	.6	73	36
35.1–40.0	42	213	5.1	.4	79	36
Hunter's Siding:						
0.1–5.0	172	20	.1	.1	6	5
5.1–10.0	189	42	.2	.1	16	7
10.1–15.0	194	85	.4	.1	21	7
15.1–20.0	108	60	.6	.2	19	8
20.1–25.0	43	19	.4	.1	33	12
Gold Center Creek:						
0.1–5.0	473	178	.4	.3	25	24
5.1–10.0	122	189	1.6	1.0	57	41
10.1–15.0	11	19	1.7	.6	73	45

[1] Infection originating in 1924 to 1928, inclusive.
[2] The plus sign signifies percentages greater than 0 but less than 0.5.

RELATIONSHIP BETWEEN INFECTION AND DAMAGE IN THE STAND AS A WHOLE

As pine infection pyramids in a stand, the percentage of trees infected increases more and more slowly relative to the increase in the number of cankers. For example, suppose that a stand exists in which all the trees are of the same size, are growing under the same environmental conditions, and are exposed to infection from distant concentrations of ribes. The resulting infection will not be absolutely uniform throughout the stand, but instead will be distributed almost at random; consequently, some of the trees will have more than one canker while others will have none. If n cankers originate and 40 percent of the trees become infected during the first year of exposure to the rust, and a second infection wave increases the number of cankers to $2n$, then the percentage of trees infected will not be increased to 80, but only to about 64. Cankers originating during the second wave will, it is true, infect approximately 40 percent of the trees, but on an average only three-fifths of this 40 percent will consist of trees becoming infected for the first time, since the infection is distributed at random through a stand in which two-fifths of the trees are already infected. In the same way, subsequent infection waves equal in magnitude to the first will successively increase the percentage of trees infected to about 78, 87, etc.

Natural stands are never composed entirely of trees of exactly the same size subjected to identical environmental conditions. In many cases, also, the infected ribes are located in or near the stand. Consequently, some of the trees are much more likely to become infected than are others, and infection is even less uniform than if it were distributed purely at random. During each successive infection wave cankers will become more numerous on those trees whose size and situation render them most liable to infection, while some of the other trees may remain rust-free for a considerable period even in localities where the disease is abundant.

Figure 3 [6] shows the probable percentages of infection corresponding to different average numbers of cankers per tree under conditions permitting perfectly random infection,[7] and under the conditions that governed the distribution of the 1927 infection on the Revelstoke

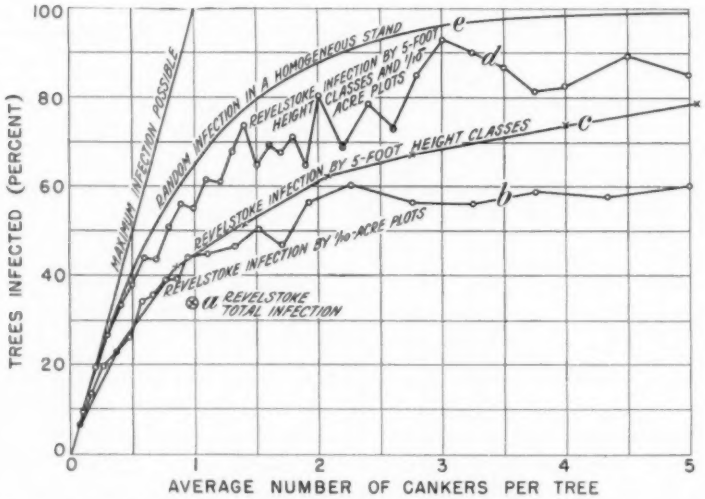

FIGURE 3.—Distribution of the 1927 infection on the Revelstoke area compared with random distribution. (Explanation in text.)

area. It will be seen that the percentage of infection on the Revelstoke area as a whole (point *a*) was far less than that which would have resulted from the same number of cankers distributed at random in a homogeneous stand of equal magnitude (curve *e*). In curve *b* the effect of differences in infection conditions (e. g., distance from ribes) has been reduced by dividing the area into 1/10-acre blocks, grouping the blocks according to the average number of cankers per tree

[6] Modified from: LACHMUND, H. G. DEGREE OF INFECTION BY CRONARTIUM RIBICOLA REQUIRED TO KILL OR SERIOUSLY INJURE TREES OF DIFFERENT SIZE CLASSES OF PINUS MONTICOLA. May 1933. [Unpublished manuscript.]

[7] This theoretical curve indicates infection probabilities in samples consisting of 10 trees each. This particular size of sample was chosen as a basis for the curve because it was of about the same general order of magnitude as most of the samples used in constructing curves *b* and *d* of fig. 3, and because it was convenient for calculation purposes. The form of the theoretical curve is affected to some extent by the size of sample for which it is drawn (e. g., where each sample consists of 10,000 trees, infection percentages are approximately 60, 84, 94, and 97 when the average numbers of cankers per tree are 1, 2, 3, and 4, respectively) but this variation is not sufficiently great to require consideration here.

(blocks containing less than four pines were not included in the computations), and plotting a separate point for each of the groups thus obtained. In curve *c* the effect of differences in tree size has been reduced by plotting a separate point for each 5-foot height class. In constructing curve *d*, separate computations were made for each 5-foot height class in each $\frac{1}{10}$-acre block (blocks containing less than four pines of any given height class were not included in the computations for that particular class), and the subsequent grouping was determined entirely by canker frequency—i. e., the data were not first averaged by blocks. In this curve the effect of differences in both tree size and infection conditions has been appreciably reduced but has not been completely eliminated, since there may be relatively great differences in size between trees of a single 5-foot height class and since infection conditions may be far from uniform even within the limits of a $\frac{1}{10}$-acre block (particularly when, as in the present instance, the ribes are located in or near the stand). Most, if not all, of the disparity between curve *d* and the theoretical curve (*e*) must therefore be ascribed to the diversity of tree sizes and situations characteristic of even the smallest units into which the basic data could practicably be organized.

For the sake of convenience the discussion in the three preceding paragraphs has been entirely confined to the relationship between total cankers present in a stand and the resultant percentage of trees infected. It is evident, however, that the same general relationship must exist between numbers of damaging cankers present and percentages of trees damaged. In the present instance, a smoothed curve drawn for the data presented in *d* of figure 3 differed algebraically by an average of only 0.14 percent from a curve constructed in the same way but based on numbers of damaging cankers and percentages of trees damaged in the various height classes and $\frac{1}{10}$-acre blocks. Curve *c* of figure 3 may therefore be considered approximately indicative of the probable distribution of damage, under damaging-canker frequencies up to an average of five per tree, in any 5-foot height class on the Revelstoke area as a whole. From these premises it is possible to estimate the approximate extent of the damage likely to be caused on this area by different given numbers of cankers. Experience with the rust at numerous infection centers has shown that waves of pine infection become progressively more severe as the increasing abundance of aeciospores results in increased ribes infection and consequent production of telia. More than 10,000 cankers became established on the Revelstoke area within relatively few years after the first appearance of the disease in this locality, and during a period when production of telia was still far below the average capacity of *Ribes lacustre* (*6*); it therefore seems entirely possible that 100,000 or more cankers might originate here in a single year during the next one or two decades even if there were no increase in the size and number of the pines. One such wave, if distributed in the same way as the 1927 infection (table 4), would result in an average of about 0.5 damaging canker per tree in the 0.1- to 5.0-foot height class and more than 4.0 damaging cankers per tree in the 25.1- to 40.0-foot height classes. Curve *c* in figure 3 indicates that the resulting damage would involve more than 25 percent of the 0.1- to 5.0-foot trees and more than 70 percent of the 25.1- to 40.0-foot trees. It is obvious

that stands of young western white pine exposed to successive infection waves of even moderate severity will be ruined for all practical purposes before commercial maturity is attained.

SUMMARY

Western white pines on several areas in British Columbia and Idaho were carefully examined for infection. A calculation, based on previous studies of the behavior of the rust on pines, was made of the probable effect on the tree of each canker. The percentage of cankers capable of damaging was found to range from nearly 100 in the smallest trees to less than 30 in trees from 45 to 50 feet tall, but the larger trees (up to at least 40 feet in height) become much more heavily infected than do small trees in the same stand and are consequently more liable to damage. Within a given height class, the percentage of cankers capable of damaging and the length of time required for such damage to occur may vary, depending on crown width, rate at which branches are being killed by suppression, and other factors.

Although complete destruction of extensive young stands can occur only when enormous numbers of cankers are present, serious damage may result from relatively few cankers, and stands exposed to even moderately severe infection will be practically destroyed before becoming commercially mature.

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THE ESSENTIALITY OF GALLIUM TO GROWTH AND REPRODUCTION OF ASPERGILLUS NIGER[1]

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INTRODUCTION

In pursuance of the same general objectives as in previous work with *Aspergillus niger* Van Tiegh.,[2] the study of the growth of this organism with highly purified nutrient components has been continued. It has resulted in the identification of the chemical element gallium as essential for the nutrition and reproduction of this fungus.

The element gallium, so far as is known to the writer, has never been claimed to be essential to the growth of an organism. Though studies of its action on growth are to be found, the experimental conditions presumably were not such as to permit any evidence of its necessity to become known.

EXPERIMENTAL METHODS

The procedures employed in previous investigations were used in these studies with certain modifications. These changes consisted in the substitution of transparent quartz vessels for similar vessels of pyrex glass. The nutrient solutions were prepared with water distilled successively in an Acree metal still, a pyrex glass still, and a transparent quartz still. The sucrose, which contained 0.0014 percent ash, was extracted with 95-percent alcohol for 6 hours in a pyrex Soxhlet extractor before use. The chemicals used were practically spectroscopically pure, usually containing only very slight impurities of sodium, calcium, and iron.

Other conditions remained unchanged. The fungus *Aspergillus niger*, "W" strain, was grown on 50 cc of a 5-percent sugar solution to which all necessary salts had been added in approximately optimum concentrations. Inoculation was made with a spore suspension. Duration of growth was 4 days at 35° C., and the cultures were filtered through 1G3 Jena glass filters. The mycelial felts were weighed after drying overnight at 103° C.

EXPERIMENTAL RESULTS

The experimental results obtained under the conditions just described are shown in table 1. Experiment 1 gave a maximum yield of 847.1 mg under conditions apparently optimum, whereas with unextracted sucrose and reagent chemicals the yield was usually approximately 1,150 mg. Tests with salts of 77 of the chemical elements led to the selection of gallium as the element capable of eliminating the decrease in yield most efficiently. Only 0.02 mg of gallium

[1] Received for publication April 11, 1938; issued November 1938.

[2] STEINBERG, ROBERT A. ROLE OF MOLYBDENUM IN THE UTILIZATION OF AMMONIUM AND NITRATE NITROGEN BY ASPERGILLUS NIGER. Jour. Agr. Research 55: 891-902. 1937.

per liter was used in experiment 2. The maximum total yield reached a value of 1,123.6 mg, or almost exactly that obtained in many previous experiments with chemicals of slightly lower purity.

The next two experiments differed only as concerned the concentrations of the trace elements added to the nutrient solutions. In the third experiment, manganese was increased to 0.05 mg per liter, and in the fourth manganese and gallium were used at 0.05 and 0.01 mg per liter, respectively. The last two experiments of this table give the results first obtained with nutrient-solution purification by use of spectroscopically pure calcium and magnesium oxides.

TABLE 1.—*Trace-element requirements of Aspergillus niger when grown for 4 days at 35° C. in quartz vessels in a nutrient solution* [1] *made with triply distilled water, extracted sucrose, and spectroscopically pure chemicals*

Element omitted	Optimum concentrations of all known constituents				Gallium added to solution				Approximately optimum concentrations of all constituents			
	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]
	Mg	*Percent*	*pH*		*Mg*	*Percent*	*pH*		*Mg*	*Percent*	*pH*	
Fe	209.7	25.76	1.73	3, bl.	409.4	38.86	1.95	4, bl.	529.1	46.57	1.82	6, bl.
Zn	20.7	2.55	2.81	2, bl.	24.0	2.28	2.67	2, bl.	25.1	2.22	2.67	2, bl.
Cu	653.1	80.24	1.71	2, t.	892.1	84.67	1.55	2, bl.	991.7	87.63	1.54	2, bl.
Mn	339.6	41.73	1.79	0	398.3	37.80	1.70	0	363.7	32.14	1.74	0
Mo	747.4	91.82	1.64	4, bl.	1,014.1	96.25	1.52	4, bl.	1,134.1	100.21	1.52	3, bl.
Ga					935.5	88.79	1.56	5, bl.	968.1	85.54	1.55	8, bl.
None	814.0	100.00	1.77	7, bl.	1,053.6	100.00	1.56	4, bl.	1,131.7	100.00	1.57	4, bl.
Max.[3]	847.1				1,123.6				1,160.9			
C. U.[4]		33.88				44.94				46.44		
pH [5]			4.85				4.94				5.00	

Element omitted	Almost optimum concentrations of all constituents				Solution purified with CaO at 100° C.				Solution purified with MgO at 100° C.			
	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]
	Mg	*Percent*	*pH*		*Mg*	*Percent*	*pH*		*Mg*	*Percent*	*pH*	
Fe	44.36	42.05	1.89	6, bl.	80.2	12.79	2.76	1, bl.	592.3	71.14	1.87	4, bl.
Zn	24.3	2.30	2.75	2, bl.	221.8	35.38	2.31	10, bl.	38.0	4.57	2.81	4, bl.
Cu	998.0	94.62	1.55	2, br.	498.6	79.53	1.94	1, bl.	781.6	91.29	1.67	4, bl.
Mn	343.2	32.54	1.80	0	195.8	31.23	2.05	0	504.2	60.57	1.76	0
Mo	1,036.5	98.26	1.53	6, bl.	660.4	105.34	1.80	8, bl.	911.2	109.45	1.65	4, bl.
Ga	848.9	80.47	1.59	6, bl.	260.0	41.47	2.30	4, bl.	658.8	79.13	1.85	4, bl.
None	1,054.8	100.00	1.60	7, bl.	626.9	100.00	1.86	6, bl.	850.5	100.00	1.73	4, bl.
Max.[3]	1,161.7				1,008.8				1,145.1			
C. U.[4]		46.47				40.35				45.80		
pH [5]			4.90				6.86				6.43	

[1] Each liter of nutrient solution contained the following ingredients: Sucrose, 50.0 g; $(NH_4)_2SO_4$, 3.40 g; KH_2PO_4, 0.55 g; Mg (as chloride), 0.025 g; iron, 0.20 mg; zinc, 0.20 mg; copper, 0.05 mg; manganese, 0.025 mg; and molybdenum, 0.02 mg. Somewhat higher concentrations of salts were used in the purification experiments.

[2] Sporulation was estimated on a scale ranging from 0 (= sterility) to 10 (= maximum sporulation); color of spores is indicated by abbreviation of the words tan, brown, and black.

[3] Maximum individual yield.

[4] Coefficient of utilization, or yield per 100 g of sucrose.

[5] Initial acidity of the nutrient solution.

TABLE 1.—*Trace-element requirements of Aspergillus niger when grown for 4 days at 35° C. in quartz vessels in a nutrient solution made with triply distilled water, extracted sucrose, and spectroscopically pure chemicals*—Continued

Element omitted	Solution unpurified				Solution purified with CaO in steamer at 100° C. and filtered				Solution purified with CaO in autoclave at 120.5° C. and filtered			
	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [2]
	Mg	*Percent*	*pH*		*Mg*	*Percent*	*pH*		*Mg*	*Percent*	*pH*	
Fe	526.5	45.26	1.81	6, bl.	72.2	8.13	2.55	1, bl.	123.8	12.31	2.36	2, bl.
Zn	22.6	1.94	2.76	2, bl.	74.9	8.43	2.59	4, bl.	153.1	15.23	2.31	8, bl.
Cu	1,056.1	90.78	1.56	2, br.	461.1	51.94	1.95	2, bl.	596.3	59.30	1.75	2, br.
Mn	369.6	31.77	1.77	0	241.5	26.07	1.94	0	221.0	21.98	2.54	0
Mo	1,061.1	91.21	1.53	4, bl.	861.6	97.03	1.63	6, bl.	1,043.2	103.75	1.51	8, bl.
Ga	926.5	79.64	1.58	4, bl.	334.6	37.68	2.17	4, bl.	431.2	42.89	2.06	5, bl.
None	1,163.3	100.00	1.54	4, bl.	887.9	100.00	1.63	6, bl.	1,005.5	100.00	1.58	8, bl.
Max.[3]	1,167.8	-------	-------	-------	1,101.7	-------	-------	-------	1,236.0	-------	-------	-------
C. U.[4]	-------	46.71	-------	-------	-------	44.07	-------	-------	-------	49.44	-------	-------
ph [5]	-------	-------	4.67	-------	-------	-------	6.64	-------	-------	-------	6.49	-------

[2] Sporulation was estimated on a scale ranging from 0 (= sterility) to 10 (= maximum sporulation); color of spores is indicated by abbreviation of the words tan, brown, and black.
[3] Maximum individual yield.
[4] Coefficient of utilization, or yield per 100 g of sucrose.
[5] Initial acidity of the nutrient solution.

The results of deficiency tests with zinc and manganese were quite good, those with copper average, and those with iron and molybdenum poor. Purification was of little effect except with gallium. Acidities at harvest differed somewhat from those obtained in previous investigations with reagent chemicals. The acidities of the minus-manganese cultures no longer tend to be higher than those of the control and the other minus cultures, though this may be associated with the exceptionally low yields. The effects of low gallium on reproduction were not marked, and seemed analogous to those of iron and zinc with respect to the extent of deficiency necessary to affect sporulation. Other untabulated experiments have demonstrated that moderate increases in iron, zinc, copper, manganese, or molybdenum were unable to overcome a deficiency in gallium.

A comparison of the effects of trace-element deficiencies on the growth of *Aspergillus* in an unpurified solution when heated at 100° C. in the steamer and when treated at 120.5° in the autoclave is given in table 1. The deficiency data are quite similar to those obtained in the previous experiments. Heating at the higher temperature did not improve the degree to which the trace elements were removed.

Several features deserve mention. The results with iron are poor despite the use of spectroscopically pure compounds, but show marked improvement as a consequence of nutrient-solution purification. Zinc results were the best yet obtained without purification, whereas purified solutions with spectroscopically pure compounds gave poorer results than purification with reagent compounds. Manganese-deficiency results likewise were superior to any yet obtained. The response to molybdenum deficiency even under these conditions is quite slight and in entire conformity with the special needs of *Asper-*

TABLE 2.—*Effect of various nitrogen compounds on trace-element requirements of Aspergillus niger when grown for 4 days at 35° C. in pyrex glass flasks in a nutrient solution [1] made with water distilled in pyrex glass, extracted sucrose, and spectroscopically pure trace elements*

Element omitted	$(NH_4)_2SO_4$				NH_4Cl (solution purified with CaO)				Urea				$NaNO_3$ (solution purified with $CaCO_3$)				NH_4Cl (solution purified with $CaCO_3$)				Urea			
	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation [1]	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation
	Mg	*Percent*	*pH*		*Mg*	*Percent*	*pH*		*Mg*	*Percent*	*pH*		*Mg*	*Percent*	*pH*		*Mg*	*Percent*	*pH*		*Mg*	*Percent*	*pH*	
Fe	308.6	31.98	2.02	6, bl.	11.4	1.51	2.74	1, bl.	154.6	20.30	2.62	1, bl.	12.7	1.46	3.16	1, bl.	12.1	1.03	2.87	0	198.2	33.21	2.69	2, bl.
Zn	30.1	3.12	2.73	2, bl.	41.5	5.50	2.70	1, bl.	136.2	17.88	2.68	3, bl.	17.1	1.96	3.31	1, bl.	20.2	1.73	2.89	0	29.2	4.90	2.77	2, bl.
Cu	804.2	83.24	1.60	6, br.	606.4	80.36	1.58	2, bl.	497.9	65.39	2.51	3, br.	614.3	70.46	3.15	4, t.	1,051.2	90.07	1.40	1, bl.	344.8	57.77	2.74	2, bl.
Mn	349.6	36.19	1.71	0	136.0	25.58	1.93	0	778.4	102.24	2.07	6, bl.	700.5	80.35	2.01	1, bl.	806.8	69.07	1.40	2, bl.	371.8	62.27	2.77	2, bl.
Mo	856.4	88.65	1.56	8, bl.	562.1	74.50	1.56	2, bl.	746.2	98.01	2.30	10, bl.	942.0	108.05	2.66	8, bl.	1,151.5	98.58	1.38	2, bl.	374.9	62.79	2.70	6, bl.
Ga	704.9	72.96	1.67	8, bl.	664.9	88.12	1.54	2, bl.	622.5	81.76	2.56	8, bl.	844.4	96.86	2.86	6, br.	1,052.8	90.13	1.38	2, bl.	232.5	38.96	2.74	2, bl.
None	966.1	100.00	1.53	7, bl.	754.5	100.00	1.49	4, bl.	761.4	100.00	2.31	10, bl.	871.8	100.00	2.89	10, bl.	1,168.1	100.00	1.40	2, bl.	596.7	100.00	2.43	8, bl.
Max.[2]	997.6				911.4				920.0				1,017.9				1,221.7				697.3			
C. U.[3]		39.90				36.46				36.80				40.72				48.87				27.89		
pH [4]			5.23				7.48				7.43				7.08				6.94				7.47	

[1] See footnote 2, table 1.
[2] See footnote 3, table 1.
[3] See footnote 4, table 1.
[4] See footnote 5, table 1.

gillus for this element with nitrate nitrogen [3] as compared with ammonium nitrogen.

A few miscellaneous experiments are shown in table 2. They were performed in the course of work now in progress to determine the extent to which quartz ware and spectroscopically pure chemicals might be dispensed with in studies on gallium. Pyrex ware and reagent chemicals, for example, were used in the experiment with ammonium sulphate together with extracted sucrose, spectroscopically pure monopotassium phosphate, and spectroscopically pure trace elements. Other experiments were performed under similar conditions except that reagent dipotassium phosphate was used. In the first experiment with urea nitrogen only reagent chemicals and unextracted sucrose were employed. Only spectroscopically pure calcium oxide was used for purification of the nutrient solution. Even these preliminary data, nevertheless, show the marked influence of gallium on the growth of *Aspergillus* under a wide range of experimental conditions. Under optimum conditions for growth the customary improvement in quantitative results should also be forthcoming.

DISCUSSION

The evidence obtained in favor of the essentiality of gallium for *Aspergillus* is quite definite, similar results having been obtained under a variety of conditions. Spectroscopically pure gallium as chloride in concentrations of 10 to 30 parts per billion aids both growth and sporulation. Salts of none of the other 76 chemical elements tested were effective to this degree at comparable concentrations. Its action, in other words, appears to be specific. The use of quartz ware and of spectroscopically pure sources of nitrogen, potassium, phosphorus, magnesium, and sulphur does not appear an essential prerequisite for positive results. Nevertheless, the use of pyrex glass vessels and reagent chemicals, even if spectroscopically pure trace elements and calcium oxide and extracted sucrose were employed, gave appreciably poorer results on gallium. The extent of the loss in yield accompanying the omission of gallium from the nutrient solution surpassed that obtained through the omission of copper, however.

The evidence obtained, therefore, in favor of the view that gallium is an essential element for *Aspergillus* is positive as respects specificity and favorable influence on growth and reproduction under a wide range of conditions. The purity of the gallium salts employed in these tests, together with the very low concentrations employed would also tend to substantiate this interpretation. That is, every known test for biological essentiality has given positive results.

The extension of these results to another organism would also be particularly desirable. Certain outstanding facts may serve as a guide in proving the necessity of gallium to other organisms. Gallium[4] is quite similar in chemical properties to aluminum and has frequently been reported as aluminum in quantitative determinations. It is widely distributed in nature in small quantities. Its absence as an impurity in aluminum salts is by no means certain, nor is its nonresponsibility for the frequently reported biologically beneficial action

[3] STEINBERG, R. A. See footnote 2.

of aluminum.[5] Some of the effects of the latter can be attributed to acidity alone, however. The reported toxicity of thallium, a chemical homologue of gallium, to tobacco by McMurtrey,[6] in association with the close duplication of the symptoms of frenching, would appear to indicate the possibility that thallium prevented the utilization of gallium by the plant. Moreover, the conditions under which this disease of the tobacco plant occurs are quite consistent with the behavior of gallium salts. A similar relation between the effects of sulphur and selenium on green plants has been reported by Hurd-Karrer.[7] The beneficial action of thallium on yeast reported by Richards[8] may also have been due to the partial substitution of thallium for the gallium needs of yeast, a well-known type of deficiency phenomenon among the fungi.

SUMMARY

Aspergillus niger was grown in quartz Erlenmeyer flasks at 35° C. for 4 days in nutrient solutions composed of sucrose extracted with alcohol; water distilled successively in an Acree metal still, a pyrex glass still, and a quartz still; and spectroscopically pure salts. The element gallium was identified as essential to the growth and development of this organism at concentrations of 0.01 to 0.02 mg per liter. Yields as low as 38 percent of maximum were obtained on its omission from the cultures. Gallium was necessary, apparently, in addition to iron, zinc, copper, manganese, and molybdenum.

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TIME INTERVAL BETWEEN CLUTCHES IN RHODE ISLAND RED PULLETS[1]

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INTRODUCTION

This paper supplements one published in 1936[2] on the same group of birds. In the first report consideration was given to the mean time interval between eggs within the clutch for the winter season and for the first laying year. This paper presents the results of a study of the mean time interval between clutches in relation to time between eggs of the clutch, clutch size, winter production, hatching-season egg weight, hatchability, persistency, and annual production.

In the previous paper the term "clutch" was used to include eggs laid on successive days, and the range in clutch size was found to vary widely in the population studied. In the work here reported a study was made of the interval between clutches throughout the first laying year. Clutches that make up any particular cycle of eggs are separated by less than 48 hours, but the interval between the last egg of a cycle and the first egg of the next cycle may be greater than 48 hours. No specific evidence is available concerning the causes of intervals of 2 to 7 days between litters in winter or of intervals of less than 30 days in spring and summer. Necessarily a rather liberal use of interval between clutches must be proposed.

CHANGES IN TIME INTERVAL BETWEEN CLUTCHES DURING THE PULLET LAYING YEAR

In calculating the time interval between clutches the cessation of laying in the birds that exhibit winter pause creates a difficulty. Since evidence had been obtained[3] that in the stock used pauses of shorter duration than 8 days are not inherited but pauses of 8 or more days are likely to depend on inheritance, it was considered advisable to omit all winter pauses of 8 days or more from November 1 to March 1. After March 1, pauses of 30 days or more probably mark the termination of the biological year and these have been omitted. All broody pauses are also omitted. The data as presented on mean monthly time interval between clutches are an approximation of normal egg laying in birds free from winter pause and in birds not undergoing annual molt within the first laying year.

[1] Received for publication February 26, 1938; issued November 1938. Contribution No. 307 of the Massachusetts Agricultural Experiment Station.

[2] HAYS, F. A. TIME INTERVAL BETWEEN EGGS OF RHODE ISLAND RED PULLETS. Jour. Agr. Research 52: 633–638, illus. 1936.

[3] HAYS, F. A. WINTER PAUSE IN RHODE ISLAND REDS. Mass. Agr. Expt. Sta. Bull. 329, 11 pp., illus. 1936.

Figure 1 indicates that pullets that are getting well started on their laying year in October are likely to lay with an interval of about 66 hours between clutches. In November and again in December there was a rapid decline in the interval between clutches. During December the shortest interval between clutches occurred. From the short interval of about 48 hours during December there was a gradual lengthening of the interval up to May. During May the interval took a very abrupt increase to about 64 hours. During June the interval showed another abrupt increase over May to about 96 hours. During July there was a slight decline to an interval of about 94 hours. In August the interval between clutches declined to 89 hours, and in

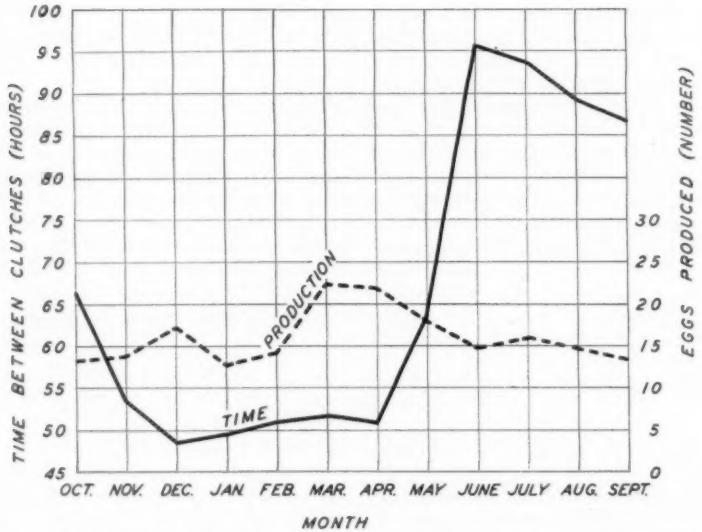

FIGURE 1.—Relation between monthly egg production and monthly interval between clutches, based on the records of 119 birds, pauses of over 8 days from November 1 to March 1 and all broody periods omitted.

September, which represents the last month of the first laying year, the interval was 87 hours.

These data show in general that when winter pause was excluded the birds were likely to lay with an interval of about 2 days between clutches during December, January, February, and March. The spring and summer seasons appeared to be characterized by intervals of 3 or 4 days between clutches. Heavy egg production and short intervals between clutches during the winter appeared to be characteristic features of production-bred flocks. In the previous report the shortest interval between eggs within the clutch occurred in April. In the data now being considered the shortest interval between clutches occurred in December.

RELATION BETWEEN MONTHLY INTERVAL BETWEEN CLUTCHES AND MONTHLY INTERVAL BETWEEN EGGS

It is desirable to know the relationship of the time interval between eggs within the clutches. These data are recorded graphically in figure 2, which presents the mean monthly interval between clutches and the mean monthly interval between eggs for the first laying year. These graphs show that short intervals between clutches were generally associated with long intervals between eggs within the clutches. For example, in February when the interval between clutches was

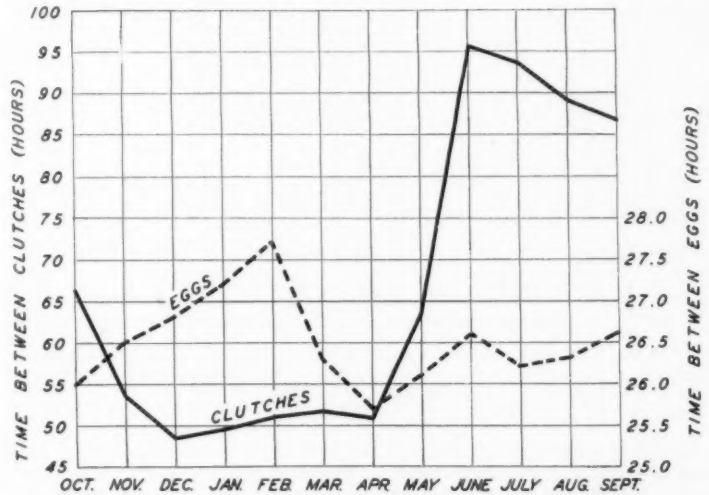

FIGURE 2.—Relation between monthly interval between clutches and monthly interval between eggs based on the records of 119 birds.

very short, the interval between eggs within the clutch reached the high level for the year. In summer when the interval between clutches increased to its greatest length, the interval between eggs remained at a relatively low level. In March, April, and May both time intervals were very short. This particular spring period offers optimum conditions for egg laying by both improved and unimproved flocks. During the winter and during the summer months when conditions are further from optimum for very intense production, birds seemed to compensate a short interval between clutches by a long interval between the eggs of a clutch or a long interval between clutches by a short interval between the eggs of a clutch. These data suggest that a true measure of intensity would involve both the interval between clutches and the interval between eggs within the clutches.

INTERVAL BETWEEN CLUTCHES IN WINTER AS RELATED TO SIZE OF WINTER CLUTCH

Data are available on 409 birds including those with winter pause and those without winter pause. After considerable study it was decided to calculate the time interval between remaining clutches after eliminating all pauses of 8 days or more between November 1 and March 1. By this method the population of 409 individuals did not approach a normal frequency distribution. In figure 3 the population is divided into nonpause and pause groups on the 8-day basis. There were 142 nonpause birds and 267 pause birds. Figure 3 indi-

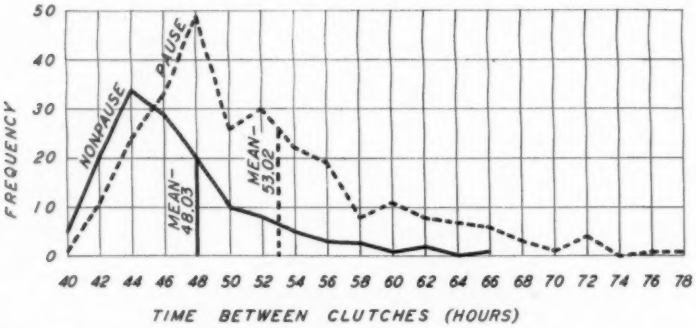

FIGURE 3.—Frequency distribution of time between clutches during winter (to March 1, 1927) for 142 nonpause birds and 267 pause birds. All pauses of 8 or more days between November 1 and March 1 are omitted in calculating time between clutches.

cates that nonpause birds required less time between clutches in the winter season than pause birds. There appeared to be two distributions with considerable overlapping, but neither distribution was sufficiently close to normal to be treated as such. Birds with winter pause were more likely to exhibit a greater time interval between clutches when they were actively laying than were nonpause birds. The variability in time interval between clutches was extremely high as compared with the variability in time interval between eggs of a clutch, as noted in the previous report.

Table 1 indicates a downward trend in clutch size with an increasing time interval between clutches in the winter season. This decline was regular and consistent until the time interval between clutches approached 65.5 hours. This particular group of 11 birds showed an unusually large mean clutch size because of three individuals that had a winter clutch size of from 5.5 to 6.5 eggs. These data therefore appear to justify the deduction that mean time intervals greater than 54 or 55 hours between clutches were likely to be associated with small clutch size or low intensity.

INTERVAL BETWEEN CLUTCHES IN WINTER AS RELATED TO WINTER EGG PRODUCTION

Mean egg production from first egg to March 1 showed generally a very consistent decline as the interval between clutches increased.

The group of 11 birds in the 65.5-hour class was an exception because of the very high winter egg record of three individuals. These data suggest that short intervals between clutches are essential to heavy winter laying.

TABLE 1.—*Time between clutches as related to winter clutch size and winter production in 394 birds*

Time between clutches in winter (hours)	Birds	Egg clutch size in winter	Winter egg production	Time between clutches in winter (hours)	Birds	Egg clutch size in winter	Winter egg production
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
41.5	19	3.56	69.71	65.5	11	2.90	59.14
44.5	75	2.96	66.70	68.5	5	1.65	37.50
47.5	99	2.74	64.79	71.5	2	6.95	70.50
50.5	63	2.83	64.87	74.5	1	1.45	15.50
53.5	53	2.68	59.08	77.5	1	1.45	15.50
56.5	32	2.54	55.19	80.5	1	2.45	85.50
59.5	17	2.39	55.50	83.5	0		
62.5	13	2.30	47.04	86.5	2	2.45	25.50

INTERVAL BETWEEN CLUTCHES IN WINTER AS RELATED TO HATCHING-SEASON EGG WEIGHT AND HATCHABILITY

There were 47 individuals that were used for breeding in the spring of 1927. Their eggs were weighed during the last week of February and the first week of March to secure an approximation of the egg weight of each bird during the hatching season extending from February 22 to April 22.

TABLE 2.—*Time interval between clutches as related to egg weight during hatching season and to hatchability of eggs from 47 birds used for breeding*

Time between clutches in winter (hours)	Birds	Egg weight during hatching season	Hatchability of eggs	Time between clutches in winter (hours)	Birds	Egg weight during hatching season	Hatchability of eggs
	Number	*Grams*	*Percent*		*Number*	*Grams*	*Percent*
41.5	5	55.5	59.5	56.5	2	55.5	35.5
44.5	15	54.8	61.9	59.6	0		
47.5	8	53.5	40.9	62.5	0		
50.5	8	52.0	47.3	65.5	2	53.5	27.8
53.5	7	53.4	56.9				

The data in table 2 show little relationship between the time interval between clutches up to March 1 and egg weight taken about March 1. There was, however, a possible suggestion of a slight decline in egg weight associated with an increased time interval between clutches.

The relation of time between clutches up to March 1 and the percentage of eggs that hatched is shown in table 2. The highest hatchability occurred in the group of 15 birds with a mean time interval of 44.5 hours between clutches. The group with the shorter interval of 41.5 hours also ranked high in hatchability. As the interval lengthened there was a tendency for hatchability to decline. Although the data are limited, there is some evidence to indicate that birds producing clutches of eggs at frequent intervals are likely to produce eggs of high hatchability.

INTERVAL BETWEEN CLUTCHES IN WINTER AS RELATED TO PERSISTENCY AND ANNUAL PRODUCTION

Persistency records covering the 365-day laying year are available on 245 birds. The data in table 3 indicate no relationship between the time interval between clutches and the duration of the laying year previous to the annual molt. The two groups of birds with a mean interval between clutches ranging from 64 to 70 hours exhibited unusually high persistency but were represented by only nine individuals and can be considered of no particular significance.

TABLE 3.—*Time between clutches as related to annual persistency and annual egg production of 245 birds*

Time between clutches during winter (hours)	Birds	Annual persistency	Annual egg production	Time between clutches during winter (hours)	Birds	Annual persistency	Annual egg production
	Number	*Days*	*Number*		*Number*	*Days*	*Number*
41.5	13	332.5	230.7	56.5	13	331.3	193.0
44.5	59	322.1	215.0	59.5	9	342.3	176.7
47.5	62	322.5	206.6	62.5	5	308.0	162.5
50.5	43	331.1	200.6	65.5	5	356.0	216.5
53.5	32	332.8	197.0	68.5	4	355.3	209.5

Annual egg production showed a definite downward trend as the time interval between clutches increased. The small group of nine birds at the bottom of the table made reasonably good annual egg records because of their very high persistency. The data in table 3 further confirm the common observation that for maximum annual records a maximum of time spent in productive laying during the winter season is essential.

SUMMARY

The mean time interval between clutches was calculated on 142 nonpause birds and on 267 pause birds up to March 1. Monthly time intervals between clutches were determined on 119 of these birds that were housed together. The relation of time interval between clutches to a number of fecundity and reproductive characters was studied. The significant relationships observed were as follows:

(1) Monthly interval between clutches attained its lowest level in December and remained at a low level from December through April. In May, June, and July the interval increased very rapidly and reached the highest level of the year in June.

(2) Although the mean time interval between clutches was short in winter after pauses had been eliminated, the level of egg production in January and February was about the same as from June to the end of the year because of winter pause.

(3) Birds with winter pause showed a greater mean time interval between clutches than nonpause birds when both were actually laying.

(4) Short intervals between clutches were associated with long intervals between eggs within the clutches, and vice versa.

(5) Small clutch size in winter was significantly associated with long time intervals between clutches.

(6) Short time intervals between clutches were associated with high winter production.

(7) There was no significant relationship between time interval between clutches and hatching-season egg weight.

(8) There was some evidence that short intervals between clutches in winter were associated with high hatchability.

(9) The time interval between clutches in winter showed no association with persistency at the close of the laying year.

(10) Short time intervals between clutches occurred in the high producers.

(11) In a breeding program short time intervals between clutches in winter should be placed along with short time intervals between eggs of a clutch as a desirable character.

RELATION BETWEEN FIBER LENGTH AND MATURITY IN COTTON [1]

By R. S. HAWKINS

Agronomist, Arizona Agricultural Experiment Station

INTRODUCTION

Plant breeders throughout the Cotton Belt are striving to increase both the length and the uniformity in length of cotton fibers. They are also interested in fiber improvement from the standpoint of maturity or wall thickness. This latter quality is less easily discernible to the naked eye than are length determinations, and if it could be safely assumed that a significant correlation exists between length and maturity, cotton-fiber improvement work would be materially simplified. The data included in this paper relate to such a correlation.

MATERIAL AND METHODS

Thirty-one samples of seed cotton were collected from as many locations in the upper Gila Valley in Arizona on October 5, 1937. This area is strictly a one-variety community growing Acala cotton. Varying availability of irrigation supplies caused wide differences in plant types, ranging from dwarfed, water-stressed plants to over-vegetative plants. The two extremes are illustrated in figure 1. One lock from each of 50 representative bolls constituted a field sample. The middle seed of each of 10 locks was placed in a Pressley sorter [2] and the percentages of fibers of various lengths were determined in the usual way. Duplicate samples were sorted in each case, making a total of 20 seeds from each field sample. In the interest of economy of time, the length classes were grouped as indicated in table 1. Such grouping probably resulted in automatically smoothing the curves in figure 2 and in making the data less variable than would have been the case if more length classes had been used.

Fiber maturity in each length class was obtained by the Shirley Institute method [3] in which the mature, intermediate, and immature fibers are counted under the microscope after treatment with an 18-percent aqueous solution of sodium hydroxide. Four samples of 100 fibers each were counted from each length class and also from the combings from each sample.

To convert the data concerning fiber maturity into a single factor, the formula $MI=\frac{10m+5t+1i}{N}$ was used. MI represents the maturity index, m the number of mature fibers in the sample, t the number of fibers intermediate in maturity, i the immature fibers, and N the total

[1] Received for publication February 16, 1938; issued November 1938.

[2] PRESSLEY, E. H. A NEW TYPE OF COTTON SORTER. Jour. Amer. Soc. Agron. 25: 89-98, illus. 1933.

[3] CLEGG, G. G. IMMATURITY OF COTTON. Empire Cotton Growing Corp. Conf. on Cotton Growing Problems, Rept. and Summary Proc. August 1930; 13-17. 1930.

number of fibers in the sample. The formula is based upon data reported by Hawkins and Serviss [4] in which it was determined that the walls of mature Acala and Pima fibers are approximately 10 times as thick as those of immature fibers. Since all gradations in fiber-wall thickness exist in the intermediate fibers, it is assumed that these

FIGURE 1.—*A*, Typical field of water-stressed cotton plants; *B*, large, overirrigated vegetative plants.

fibers average midway between the mature and immature fibers in wall thickness. The thickening of fiber walls is a biological process effected by hereditary and environmental influences. The maturity index as used in this paper is a measure of these influences on the average thickness of the fiber walls in a given sample.

[4] HAWKINS, R. S., and SERVISS, GEORGE H. DEVELOPMENT OF FIBERS IN THE PIMA AND ACALA VARIETIES. Jour. Agr. Research 40: 1017-1029, illus. 1930.

EXPERIMENTAL RESULTS

The maturity indices for the fiber by length classes of the 31 samples of cotton, together with the percentages of fibers included in each length class, are given in table 1. A small proportion of the fibers becomes detached from the seed during the combing process prior to placement in the sorter. The length of fiber distribution made from combings indicates that usually the fiber lengths that make up the combings are approximately in the same proportion as in the sample proper. However, the combings contain more immature fibers than any of the length classes as shown by the data in table 1. This is not surprising, since immature fibers are weaker and more easily detached or broken than mature ones.

TABLE 1.—*Maturity indices and percentages of cotton fibers in relation to length classes*

Field sample No.	Combings [1]		Four-eighths and five-eighths inch		Six-eighths and seven-eighths inch		Eight-eighths and nine-eighths inches		Ten-eighths inches		Weighted average maturity index for sample
	Maturity index	Percent	Maturity index	Percent	Maturity index	Percent	Maturity index	Percent	Maturity index	Percent	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
1	0.277	31.4	0.251	21.3	0.375	19.0	0.383	25.6	0.403	2.8	0.321
2	.389	10.7	.545	9.3	.483	25.7	.584	48.4	.427	5.8	.524
3	.397	5.8	.462	10.2	.570	30.6	.611	48.8	.409	4.7	.562
4	.521	10.0	.510	10.3	.568	22.4	.581	49.9	.453	7.4	.553
5	.476	10.6	.507	10.0	.552	21.8	.654	52.1	.463	5.5	.588
6	.523	10.0	.583	11.6	.609	21.8	.631	51.5	.485	5.2	.603
7	.444	7.5	.561	9.4	.579	20.0	.536	55.7	.654	7.4	.549
8	.464	15.5	.468	13.3	.540	24.4	.529	38.3	.578	8.4	.517
9	.495	6.4	.594	11.2	.547	19.6	.617	55.3	.479	7.5	.583
10	.426	7.6	.578	14.2	.597	34.3	.602	41.1	.462	2.8	.580
11	.398	5.5	.570	14.9	.568	33.3	.602	42.0	.479	4.2	.569
12	.538	11.9	.515	14.8	.691	33.1	.629	37.7	.375	2.5	.615
13	.464	15.8	.515	16.4	.611	35.6	.646	30.9	.365	1.2	.579
14	.413	10.2	.543	10.5	.535	16.6	.393	55.8	.364	7.0	.433
15	.523	13.6	.562	15.2	.637	31.6	.684	37.8	.549	1.9	.627
16	.424	7.5	.549	12.3	.578	21.8	.519	50.5	.502	8.0	.528
17	.426	10.8	.469	8.6	.536	17.3	.568	54.6	.355	8.6	.520
18	.448	13.0	.632	17.7	.539	30.8	.523	33.4	.463	5.0	.534
19	.522	9.4	.601	12.7	.636	29.1	.715	44.1	.702	4.7	.659
20	.526	8.0	.559	9.6	.634	20.5	.634	54.1	.481	7.8	.602
21	.500	10.8	.591	10.1	.600	20.9	.622	53.0	.543	5.2	.597
22	.543	10.9	.583	9.4	.559	21.0	.646	52.9	.496	5.7	.601
23	.625	7.0	.562	10.7	.646	24.8	.580	52.9	.430	4.6	.591
24	.393	12.3	.538	12.5	.559	21.7	.518	46.6	.450	6.9	.509
25	.450	13.6	.502	13.7	.577	24.7	.567	42.8	.499	5.2	.541
26	.474	12.0	.589	13.5	.666	19.9	.611	48.6	.526	5.9	.597
27	.664	11.9	.522	9.2	.661	28.6	.644	42.3	.607	8.0	.623
28	.547	11.3	.516	11.6	.599	14.9	.635	49.9	.629	12.2	.605
29	.522	8.2	.607	11.0	.578	16.6	.725	55.8	.542	8.4	.656
30	.349	12.5	.450	15.5	.515	26.2	.436	38.9	.433	6.8	.474
31	.481	7.6	.639	13.3	.667	28.3	.617	43.7	.623	7.0	.624
Mean	.472	10.95	.538	12.39	.581	24.42	.588	46.29	.491	5.95	.563

Mean difference of the differences between (4) and (6), 0.060; between (6) and (8), 0.007; between (8) and (10), −0.094. and error of mean differences ±0.008 [2], ±0.011, and ±0.017 [2].

[1] Percentages are based on fibers four-eighths of an inch or longer, assuming that most of the shorter fibers go into waste.

[2] Highly significant differences, as calculated by Student's *t* method, the chances being greater than 99 to 1 that the differences are not due to chance. (See text for more detailed explanation.)

The longest fibers, those of the 1⅛-inch class, were more immature than fibers of any other class, having a low average maturity index of 0.491. However, this length class contained only 5.95 percent of the fibers, a materially lower percentage than any other length class.

The best fibers from the standpoint of maturity were in the ⅝- and ¾-inch class with a maturity index of 0.588, although there was no significant difference between this class and the ⅝ and ⅞-inch class, which had a maturity index of 0.581. The former class contained almost half of the total fibers, 46 percent, and these two classes comprised more than 70 percent of the total fibers. The shortest fibers considered, the ½- and ⅝-inch class, had a maturity index of 0.538, which was materially better than that of the longest fibers but inferior to that of the intermediate classes.

Statistical analysis by means of Student's *t* method, as described by Fisher,[5] shows that the differences in maturity between the short, intermediate, and long length classes are highly significant, the chances being greater than 99 to 1 that these differences are not due to chance.

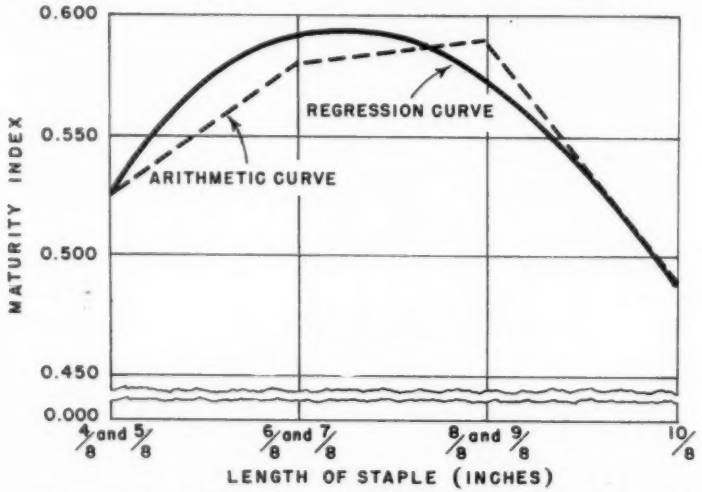

FIGURE 2.—Relation between maturity and length of staple as calculated from table 1. (Method described by LOVE, HARRY H. APPLICATIONS OF STATISTICAL METHODS TO AGRICULTURAL RESEARCH. 501 pp., illus. Shanghai. 1936.)

According to Student's *t* method, when the quotient of the mean differences divided by the error of the mean differences falls between 2.042 and 2.750 (with 31 samples involved as in this particular problem), the data show significant differences. No significance is shown when the quotient is lower than 2.042, and when higher than 2.750 the differences are highly significant. Thus the mean of the differences between the maturity indices in columns 4 and 6 is 0.060 and when divided by the error of the mean difference, 0.008, gives a quotient which is very much greater than 2.750 and therefore is highly significant. Similarly, the differences between columns 6 and 8 are not significant, while those between columns 8 and 10 are highly significant. These significant differences strengthen the evidence that there is a significant second-degree curve relationship between maturity and fiber length as shown by the close fitting seen in figure 2.

[5] FISHER, RONALD AYLMER. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, rev. and enl., 283 pp., illus. Edinburgh and London. 1930.

The curve respresenting the arithmetic average maturity of the different length classes and the regression curve from the same data are closely related.

The wide variations in soil moisture, coupled with the resultant differences in plant types prevailing in the fields from which the cotton samples were taken, add greatly to the conclusiveness of the results. These fields were not under observation, except on the day of sampling; hence, available data do not permit correlation between seasonal environmental plant growth influences and fiber maturity.

CONCLUSIONS

The longest and also the shortest fibers, in a given lot of cotton, are less mature than the fibers of intermediate lengths.

Fibers toward the top of the range in intermediate lengths are slightly better in maturity than those toward the bottom. Cotton breeders who select progenies with greater proportions of the longer intermediate fibers, unconsciously or otherwise, select toward improvement in fiber maturity.

A MOSAIC DISEASE OF TURNIP [1]

By C. M. TOMPKINS

Assistant plant pathologist, California Agricultural Experiment Station [2]

INTRODUCTION

A mosaic disease of turnip (*Brassica rapa* L.) occurs on Long Island, N. Y. Typical specimens of this disease were obtained for comparative greenhouse studies with virus diseases affecting certain cultivated crucifers in California (*15*, *16*, *17*, *18*).[3]

It is the purpose of this paper to describe briefly the symptoms of this mosaic disease of turnip from New York, to present experimental evidence on transmission, host range, and properties of the virus, and to discuss its possible relationship to other crucifer viruses.

REVIEW OF LITERATURE

In reviewing the literature, it is apparent in some instances that the names, "turnip mosaic" and "rutabaga mosaic," have been used synonymously. It has seemed advisable, therefore, to include in this review all references pertaining to rutabaga mosaic, as well as to turnip mosaic, irrespective of the identity of the particular virus involved.

In 1921, Gardner and Kendrick (*4*) described a mosaic disease of turnip that was found in Indiana. The causal virus was transmitted to healthy turnip seedlings by rubbing wounded plant parts with cotton soaked in extracted juice from diseased plants. The incubation period ranged from 16 to 26 days. Unsuccessful attempts were made to infect red and white varieties of radish (*Raphanus sativus* L.).

What appears to have been the same disease on turnip, Chinese cabbage (*Brassica pe-tsai* Bailey), and mustard (*B. japonica* Coss) was also described by Schultz (*12*). By means of the leaf-mutilation, rubbing method of inoculation, healthy turnip, Chinese cabbage, and mustard seedlings were infected with extracted juice from the corresponding diseased plants, in 20 to 30 days. Further, when the green peach aphid (*Myzus persicae* (Sulzer)) was transferred to healthy turnip and mustard seedlings after having fed on diseased turnip, Chinese cabbage, and mustard plants, mosaic mottling developed in 12 to 20 days. Evidence for seed transmission was negative, since mustard seed from mosaic plants yielded healthy seedlings.

Gram (*5*) found a mosaic disease of turnip in eight localities in Denmark in 1921. Other plants susceptible to natural infection included swedes or rutabaga (*Brassica campestris* L. var. *napo-brassica* DC.), radish, and charlock (*Sinapsis arvensis* L.).

In a brief report by Thatcher (*14*), reference is made to inoculation experiments which showed that mustard, rutabaga, flat turnip, and

[1] Received for publication February 16, 1938; issued November 1938.

[2] The writer is indebted to Dr. M. W. Gardner for advice and assistance and to Prof. B. A. Madson and W. W. Mackie for providing greenhouse space and facilities. Valuable assistance in the greenhouse work was rendered by employees of the Federal Works Progress Administration.

[3] Italic numbers in parentheses refer to Literature Cited, p. 601.

Chinese cabbage are very susceptible to mosaic. Rape was considered to be less susceptible. The identity of this crucifer virus was not disclosed.

Apparently the preceding citation by Thatcher served merely as a preliminary announcement of a more detailed report on studies of a mosaic disease of crucifers conducted by Clayton (*2*). A mosaic disease of rutabaga was found to be common but not serious on Long Island, N. Y. It was also observed on Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* DC.), cauliflower (*B. oleracea* L. var. *botrytis* L.), and black mustard (*B. nigra* Koch), but not on white turnip. Infection experiments involved the use of the leaf mutilation, rubbing method of inoculation and of the cabbage aphid (*Brevicoryne brassicae* (L.)) and the green peach aphid. The cabbage aphid proved very effective as a vector, but the green peach aphid gave negative results. An incubation period of 3 to 5 weeks was reported. Symptoms of the disease were favored by relatively high temperatures (70° to 80° F.), while lower temperatures (55° to 65°) induced masking. White mustard (*B. alba* Rabenh.), black mustard, Chinese cabbage, turnip, rutabaga, and rape (*B. napus* L.) were susceptible to artificial infection. Brussels sprouts and cauliflower were difficult to infect. Although cabbage (*B. oleracea* L. var. *capitata* L.) was considered to be highly resistant or immune to the disease, the suggestion was advanced that it might act as a carrier. No evidence of seed transmission was obtained. Apparently Clayton did not recognize any differences between the rutabaga mosaic virus with which he worked and the turnip mosaic virus reported elsewhere (*4*, *5*, *12*).

A mosaic disease of Chinese cabbage, turnip, and mustard occasionally caused 30 percent loss near Fukuoka, Japan, according to Takimoto (*13*). The virus was transmitted by mechanical inoculation to cauliflower but not to cabbage or radish. Aphids (species not given) were held responsible for natural transmission of the disease. Takimoto considered the disease to be similar to those described by Gardner and Kendrick (*4*), Schultz (*12*), and Clayton (*2*).

Samuel (*11*) reported on turnip mosaic in Australia in 1931. A mosaic disease of turnip and rutabaga was reported from Florida by Weber (*19*) in 1932 and from Japan by Hino (*6*) in 1933.

Edson, Miller, and Wood [4] recorded the occurrence of rutabaga mosaic in Connecticut and turnip mosaic in Mississippi in 1934.

A virus disease of cabbage, mustard, turnip, and horseradish (*Radicula armoracia* Robins) was described by Hoggan and Johnson (*7*) in 1935. The virus was transmitted by mechanical inoculation and by the cabbage and green peach aphids to cabbage, black mustard, turnip, tobacco (*Nicotiana tabacum* L.) var. Connecticut Havana No. 38, *N. glutinosa* L., the hybrid *N. tabacum N. glutinosa*, currant tomato (*Lycopersicum pimpinellifolium* Dunal), and spinach (*Spinacia oleracea* L.) var. Bloomsdale. The temperature range favorable to infection was 70° to 80° F. Properties of the virus were given: Thermal death point, 54° C. for a 10-minute exposure; longevity in vitro, less than 3 days at 20°–22°; tolerance to dilution, 1 to 1,000.

[4] EDSON, H. A., MILLER, PAUL R., and WOOD, JESSIE I. DISEASES OF PLANTS IN THE UNITED STATES IN 1934. U. S. Bur. Plant Indus., Plant Disease Reptr. Sup. 90: 75. 1935.

Losses caused by turnip mosaic in Germany, according to Pape (*9*), may vary from 1 to 90 percent. Some varieties of turnip were found to be much more susceptible to infection than others. He reported transmission of the virus to healthy turnip plants by mechanical inoculation and by means of the tarnished plant bug (*Lygus pratensis* (L.)).

Chamberlain (*1*) recognized turnip mosaic on rape plants at Palmerston North, New Zealand, in 1932 and subsequently on rutabagas, rape, and turnips in the same and other districts. He obtained mechanical transmission of the disease by the rubbing method and by means of the cabbage and green peach aphids which commonly occur on cruciferous crops in New Zealand, but failed to give the time required in either case for symptom expression. Tests for seed transmission involved planting seeds derived from mosaic-infected rutabaga plants. Of 432 seedlings, none showed symptoms of mosaic. In host-range studies, no infection, by means of artificial inoculation, was secured on cabbage, cauliflower, Brussels sprouts, broccoli (*Brassica oleracea* L. var. *botrytis* L.), and radish. However, all of these crucifers, except radish, were infected when the green peach aphid served as the vector. Brown necrotic lesions were obtained on tobacco (variety not specified). Annual stock (*Matthiola incana* R. Br. var. *annua* Voss) is a natural host for the turnip mosaic virus which induces flower breaking as well as leaf mottling.[5] Other natural hosts include wallflower (*Cheiranthus cheiri* L.) and a number of common cruciferous weeds. In tests for varietal resistance, one variety of turnip proved highly resistant and several others less so. Recommendations for control of the disease in seed crops were dipping the leaves of plants at transplanting time in a nicotine solution in order to kill the insect vectors, rogueing of all infected plants, avoidance of other cruciferous crops and volunteer seedlings, and, when mosaic appears, spraying the plants with nicotine solution.

Edson and Wood[6] listed turnip mosaic as prevalent in Connecticut in 1935.

In 1936, Kaufmann (*8*) described a virus disease of winter-sown rape (*Brassica rapa* L. var. *oleifera* Delile), rutabaga, and colza (*B. napus* L. var. *oleifera* Delile). Infection of healthy rape, rutabaga, and colza plants resulted from juice inoculations and when the tarnished plant bug was used, both in the greenhouse and in the field. Significant losses were observed only on rutabaga. The identity of this virus was apparently not clearly established.

SYMPTOMS OF THE DISEASE

Studies of the symptoms caused by the turnip mosaic virus from New York have been limited to greenhouse observations. Initial symptoms of the disease on leaves of Purple Top White Globe turnip seedlings consist of a conspicuous, coarse, systemic clearing of the veins, with interveinal mottling, which collectively impart a yellowish caste. The leaves show marked crinkling and slight dwarfing (fig.

[5] Letter dated July 2, 1937, from E. E. Chamberlain, Plant Diseases Division, Plant Research Bureau, Palmerston North, New Zealand.

[6] EDSON, H. A., and WOOD, JESSIE I. DISEASES OF PLANTS IN THE UNITED STATES IN 1935. U. S. Bur. Plant Indus., Plant Disease Reptr. Sup. 96: 200. 1936.

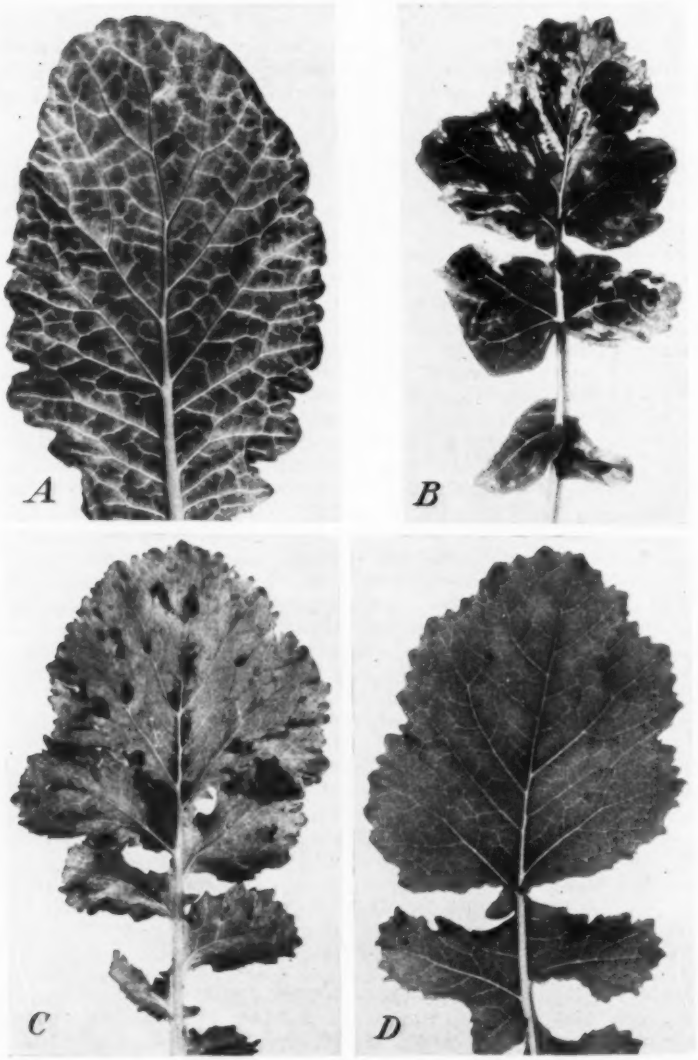

FIGURE 1.—Symptoms produced by the turnip mosaic virus on Purple Top White Globe turnip leaves after artificial inoculation in the greenhouse at 13°–19° C.: *A*, Early symptoms consist of coarse, yellow vein clearing and mottling; *B*, intermediate symptoms consisting of dark-green tissue with numerous raised islands of irregular shape and a small amount of chlorotic tissue; *C*, late symptoms in which the leaf has become very chlorotic with only a few dark-green, raised islands; *D*, noninoculated control.

1, *A*), when contrasted with normal, healthy specimens. Stunting of the plants is also apparent in the early stages of infection. Gradually, as the leaves expand, the coarse, yellow appearance is replaced by a preponderance of very dark-green, irregular, raised islands, interspersed with a restricted amount of chlorotic tissue (fig. 1, *B*). In later stages of infection, the dark-green islands are almost entirely replaced by chlorotic, light-green areas (fig. 1, *C*). Crinkling of the leaves is accentuated in the latter two stages of infection (fig. 1, *B*, *C*) in contrast to the normal, healthy leaf (fig. 1, *D*). Occasionally in some infected plants the leaves were unsymmeterical. Although the turnip mosaic virus causes severe stunting of turnip plants, no lethal effect has been observed on this or any other susceptible host.

MATERIALS AND METHODS

In 1934, 12 white turnip stecklings showing pronounced symptoms of mosaic infection were collected in a commercial planting near Mattituck, Long Island, N. Y., and forwarded to Berkeley, Calif. Within several weeks after transplanting, these stecklings produced new leaves, all of which were mottled. Transfers were then made to healthy Purple Top White Globe turnip seedlings by mechanical inoculation. The virulence of, and the symptoms produced by, this virus have remained unchanged through successive monthly transfers since 1934. Purple Top White Globe turnip seedlings were used as the standard test plant for recovery of the virus from infected plants and for property studies.

All inoculations were made in a greenhouse where temperatures ranged from 13° to 19° C. The methods followed were essentially those described in recent papers (*16*, *18*). Mechanical inoculations were made by dusting the leaves with 600-mesh, powdered carborundum (*10*) and lightly rubbing with absorbent cotton dipped in juice from a diseased plant.

EXPERIMENTAL RESULTS

TRANSMISSION

The turnip mosaic virus can be transferred to healthy turnip seedlings by mechanical inoculation with or without carborundum (*10*), but the percentage of infection is reduced approximately 50 percent when this abrasive is omitted. As a result of numerous tests, the incubation period was determined as ranging from 13 to 21 days.

Clayton (*2*) used both the cabbage aphid and the green peach aphid in his insect transmission studies with the rutabaga mosaic virus, finding that the latter was much less efficient. Since Essig (*3*) found that the cabbage and green peach aphids breed naturally on cultivated and wild crucifers in California and other Western States and because the New York turnip mosaic virus may eventually be found in California, it seemed desirable to test the two aphid species under greenhouse conditions.

Following the procedure outlined in a recent paper (*18*), noninfective cabbage and green peach aphids[7] were fed on recently infected

[7] Noninfective cabbage and green peach aphids, previously identified as such by E. O. Essig, were kindly supplied by H. H. P. Severin and J. H. Freitag, Division of Entomology and Parasitology, University of California.

turnip plants for 24 to 48 hours, after which they were transferred in lots of approximately 20 aphids each to healthy turnip seedings. After 24 hours, all plants were sprayed with nicotine sulphate solution. Healthy turnip seedlings infested with noninfective aphids and noninoculated plants free from aphids served as controls.

Of 20 turnip plants infested with viruliferous cabbage aphids, 16 showed typical symptoms of the disease in 15 to 17 days, while 4 plants damped off. When infective green peach aphids were placed on 20 turnip plants, 100-percent infection occurred, the incubation period ranging from 12 to 18 days. The virus was recovered from all infected plants by mechanical inoculation. None of the controls became diseased. Other possible means of transmission were not investigated.

EXPERIMENTAL HOST RANGE

Young seedlings of various turnip and rutabaga varieties were tested by mechanical inoculation, with a suitable number of plants for controls. The following varieties of turnip proved to be highly susceptible: Amber or Yellow Globe, Bortfelder, Cowhorn, Early Purple Top Milan, Early White Flat Dutch, Extra Early White Milan, Large White Norfolk, Orange Jelly or Golden Ball, Purple Strap Leaf, Purple Top Strap Leaved Early, Purple Top Yellow Aberdeen, Seven Top, Shogoin or Japanese, Snowball, Southern Prize, White Egg, and White Milan. Of rutabaga varieties, the following varieties proved to be highly susceptible: American Purple Top, Hartleys Bronze Top, Large White, and Monarch or Tankard. The variety Long Island Improved was only slightly susceptible to infection.

By means of mechanical inoculation, the turnip mosaic virus from New York was transmitted to 18 species of plants representing 12 genera in 6 families, as follows:

Cruciferae:
- Cabbage (*Brassica oleracea* L. var. *capitata* L.).
- Cauliflower (*B. oleracea* var. *botrytis* L.).
- Rutabaga (*B. campestris* L. var. *napo-brassica* DC.).
- Turnip (*B. rapa* L.).
- Leaf or Chinese mustard (*B. juncea* Coss).
- Chinese cabbage (*B. pe-tsai* Bailey).
- Wild yellow mustard (*B. campestris* L.).
- *B. adpressa* Boiss.
- Shepherds-purse (*Capsella bursa-pastoris* Medic.).
- Annual stock (*Matthiola incana* R. Br. var. *annua* Voss).
- Dames violet (*Hesperis matronalis* L.).
- Virginian stock (*Malcomia maritima* R. Br.).
- Honesty (*Lunaria annua* L.).
- Chinese radish (*Raphanus sativus* L. var. *longipinnatus* Bailey).

Chenopodiaceae: Lambsquarters or white pigweed (*Chenopodium album* L.)
Resedaceae: Mignonette (*Reseda odorata* L.).
Begoniaceae: Fibrous-rooted begonia (*Begonia semperflorens* Link and Otto).
Verbenaceae: Garden verbena (*Verbena hybrida* Voss).
Solanaceae:
- Turkish tobacco (*Nicotiana tabacum* L.).
- *N. glutinosa* L.
- Petunia (*Petunia hybrida* Hort.).

In the family Cruciferae, all susceptible plants showed systemic infection. These include Winter Colma cabbage (fig. 2) with numerous chlorotic lesions which later became necrotic around the edges; February, April, Danish Perfection, Dryweather Danish Giant,

Early March, Early Snowball, and Super Snowball cauliflower with chlorotic to yellow lesions; Long Island Improved rutabaga with chlorotic lesions; Flat Turnip and Florida Broadleaf varieties of leaf or Chinese mustard with leaf mottling and rugosity; pe-tsai with coarse yellow vein banding and leaf distortion; *Brassica adpressa*, wild yellow mustard, and shepherds-purse with coarse mottling; Fiery Blood Red annual stock with coarse vein clearing, diffuse mottling, and occasional midrib distortion of leaves and flower breaking; dames violet with a fine type of mottling followed by small

FIGURE 2.—Early symptoms, consisting of small, diffuse, chlorotic rings, on Winter Colma cabbage leaf 27 days after artificial inoculation with the turnip mosaic virus in the greenhouse at 13° to 19° C.

necrotic lesions, raised dark-green islands, and marked bleaching; and Virginian stock, honesty, and Chinese radish with mottling.

Infection of lambsquarters or white pigweed was indicated by local, yellow lesions on inoculated leaves only. Later, these became necrotic, with some coalescence. Pronounced mottling of leaves characterized infection of mignonette, garden verbena, and petunia. Small chlorotic lesions appeared on leaves of infected fibrous-rooted begonia. Local chlorotic lesions which later became necrotic occurred only on inoculated leaves of Turkish tobacco and *Nicotiana glutinosa* (fig. 3). The virus was recovered from all infected plants except from lambsquarters, mignonette, and fibrous-rooted begonia.

No infection was obtained by mechanical inoculation in 53 species of plants representing 43 genera in 23 families, as follows:

Cruciferae:
Kale (*Brassica oleracea* L. var. *acephala* DC.).
Brussels sprouts (*B. oleracea* var. *gemmifera* DC.).
Sprouting broccoli (*B. oleracea* var. *botrytis* L.).
Kohlrabi (*B. oleracea* var. *caulorapa* DC.).
Rape (*B. napus* L.).
Black mustard (*B. nigra* Koch).
White mustard (*B. alba* Rabenh.).
Charlock (*B. arvensis* (L.) Ktze.).
B. integrifolia O. E. Schulz var. *chevalieri* R. Porteres.
Wallflower (*Cheiranthus cheiri* L.).
Evening scented stock (*Matthiola bicornis* DC.).

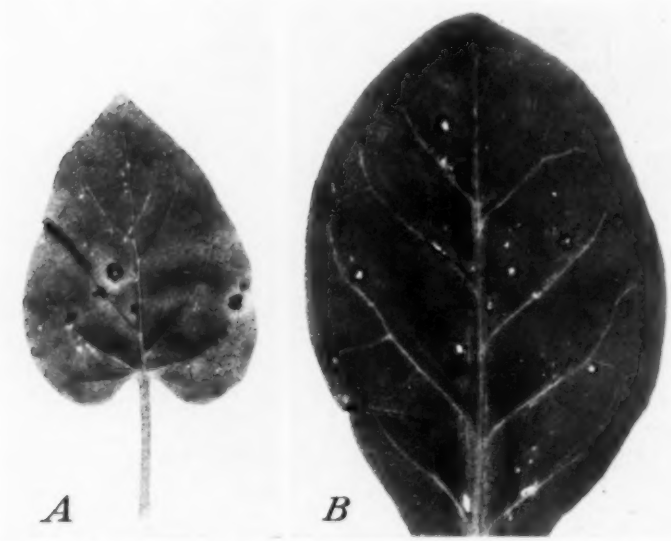

FIGURE 3.—Symptoms produced by the turnip mosaic virus by artificial inoculation in the greenhouse at 13° to 19° C.: *A*, Local chlorotic lesions on inoculated leaf of *Nicotiana glutinosa* which later became necrotic with tan centers; *B*, local necrotic lesions on leaf of *N. tabacum* var. Turkish.

Sweet alyssum (*Alyssum maritimum* Lam.).
Radish (*Raphanus sativus* L.) var. White Icicle and Crimson Giant Forcing.
Gramineae: Corn (*Zea mays* L.) var. Golden Bantam.
Polygonaceae: Rhubarb (*Rheum rhaponticum* L.).
Chenopodiaceae:
Sowbane or nettleleaf goosefoot (*Chenopodium murale* L.).
Spinach (*Spinacea oleracea* L.) var. Bloomsdale.
Caryophyllaceae:
Sweet-william (*Dianthus barbatus* L.).
Babysbreath (*Gypsophila panicualta* L.).
Ranunculaceae: Rocket larkspur (*Delphinium ajacis* L.).
Rosaceae: *Geum chiloense* Balb.
Leguminosae:
Garden pea (*Pisum sativum* L.) var. Alderman.
Broadbean (*Vicia faba* L.).

Tropaeolaceae: Garden nasturtium (*Tropaeolum majus* L.).
Euphorbiaceae: Castor-bean (*Ricinus communis* L.).
Violaceae: Pansy (*Viola tricolor* L.).
Onagraceae:
Clarkia elegans Dougl.
Godetia grandiflora Lindl.
Umbelliferae: Celery (*Apium graveolens* L.) var. Golden Self Blanching.
Plumbaginaceae: Everlasting or sea-lavender (*Statice latifolium* Kuntze).
Boraginaceae:
Forget-me-not (*Myosotis alpestris* Schmidt).
Common heliotrope (*Heliotropium peruvianum* L.).
Labiatae: Flowering sage (*Salvia farinacea* Benth.).
Solanaceae:
Solanum aviculare Forst.
Potato (*S. tuberosum* L.).
Tomato (*Lycopersicum esculentum* Mill. var. *vulgare* Bailey) var. Early Santa Clara Canner.
Currant tomato (*L. pimpinellifolium* Dunal).
Nicotiana langsdorfii Weinm.
N. rustica L.. var. *humulis* Schrank.
Tobacco (*N. tabacum* L.) var. White Burley.
Jimsonweed (*Datura stramonium* L.).
Scrophulariaceae:
Snapdragon (*Antirrhinum majus* L.).
Penstemon or beardtongue (*Penstemon barbatus* Nutt.).
Dipsaceae: Mourning bride or pincushion flower (*Scabiosa atropurpurea.*).
Cucurbitaceae: Cucumber (*Cucumis sativus* L.).
Campanulaceae: Canterbury-bells (*Campanula medium* L.).
Lobeliaceae: *Lobelia hybrida* Hort.
Compositae:
Head lettuce (*Lactuca sativa* L. var. *capitata* Hort.) var. New York and Tom Thumb.
Dandelion(*Taraxacum officinale* Weber).
Annual marguerite (*Chrysanthemum coronarium* L.).
English daisy (*Bellis perennis* L.).
African marigold (*Tagetes erecta* L.).
French marigold (*T. patula* L.).
Winter Cape-marigold (*Dimorphotheca aurantiaca* DC.).
Gaillardia pulchella Foug. var. *picta* Gray.
Cineraria (*Senecio cruentus* DC.).

Subsequent inoculations to turnip with extracted juice from inoculated plants of the above-mentioned plant species failed to cause infection.

PROPERTIES OF THE VIRUS

Determinations were made on longevity in vitro, inactivation temperature, and tolerance to dilution, and the results are given in table 1.

Virus samples consisted of 2 cc of undiluted juice, from recently infected turnip plants, in small, thin-walled test tubes. In determining resistance to aging in vitro, samples were stored at a constant temperature of 22° C. Resistance to heating was determined by heating virus samples in a thermostatically controlled water bath for 10 minutes. Virus dilutions were made with distilled water. Immediately following a specific treatment, young Purple Top White Globe turnip plants were then tested for infectivity by mechanical inoculation.

The turnip mosaic virus was found to be infective for 48 hours after aging in vitro, but was inactivated after 72 hours. The virus was infectious after heating for 10 minutes at various temperatures not exceeding 60° C., but was inactivated at 63° C. A dilution tolerance

of 1:3,000 was established. In each of the three tests, 25 noninoculated turnip plants served as controls and they continued healthy throughout.

TABLE 1.—*Longevity in vitro, inactivation temperature, and tolerance to dilution of the turnip mosaic virus*

[Five trials on 25 plants in all cases]

LONGEVITY IN VITRO, 22° C.

Age (hours)	Plants infected	Age (hours)	Plants infected
	Number		*Number*
0	25	96	0
24	13	120	0
48	2	144	0
72	0	168	0

INACTIVATION TEMPERATURE (10 MINUTES)

Temperature (°C.)	Plants infected	Temperature (°C.)	Plants infected
	Number		*Number*
50	17	65	0
55	10	70	0
60	2	(1)	21
63	0		

TOLERANCE TO DILUTION

Dilution	Plants infected	Dilution	Plants infected
	Number		*Number*
0	25	1:2,000	4
1:10	20	1:3,000	4
1:100	17	1:4,000	0
1:500	7	1:5,000	0
1:1,000	8		

[1] Not treated.

DESCRIPTION OF THE TURNIP MOSAIC VIRUS FROM NEW YORK

Transmitted in greenhouse tests by means of the cabbage aphid (*Brevicoryne brassicae*) and the green peach aphid (*Myzus persicae*). Transmissible by mechanical inoculation, using expressed juice with or without powdered carborundum. Incubation period 13 to 21 days when Purple Top White Globe turnips were used. Resistance to aging in vitro between 2 and 3 days. Inactivation temperature between 60° and 63° C. for 10-minute exposure. Tolerance to dilution approximately 1 to 3,000. White turnip (*Brassica rapa* L.) and certain other vegetable and ornamental crucifers are susceptible. On turnip, early symptoms consist of diffuse mottling of the leaves; later, irregular-shaped, raised dark-green islands are scattered on a markedly chlorotic background. Slight distortion and rugosity of leaves in late stages of infection. Local necrotic lesions produced on *Nicotiana glutinosa* and *N. tabacum*. Chinese radish susceptible, but not ordinary radish.

COMPARISON OF THE TURNIP MOSAIC VIRUS FROM NEW YORK WITH CERTAIN OTHER CRUCIFER VIRUSES

In a recent paper (*16*) the writer briefly compared the symptoms produced on certain cultivated crucifers by the cauliflower mosaic and turnip mosaic (from New York) viruses. When sprouting

broccoli, cabbage, cauliflower, kohlrabi, radish, and turnip seedlings were inoculated with the cauliflower mosaic virus, early symptoms were manifested by vein clearing (*16, fig. 3, C*; fig. 1, *A*) (fig. 4). The turnip mosaic virus, however, produced different symptoms on infected plants: Cabbage, chlorotic rings later becoming necrotic; cauliflower, pale-green to yellow lesions (*16, fig. 5, A, B*). As shown in this paper, a coarse leaf mottle characterizes infection of turnip. Sprouting broccoli, kohlrabi, and radish were not infected by the turnip mosaic virus. On annual stock, the cauliflower mosaic virus caused a coarse vein clearing of the leaves (*16, fig. 4, A, B, C*) but not flower breaking; the turnip mosaic virus induced not only coarse vein clear-

FIGURE 4.—Vein-clearing symptoms produced by the cauliflower mosaic virus on Purple Top White Globe turnip leaf by artificial inoculation in the greenhouse at 13° to 19° C.

ing and diffuse mottling but also flower breaking. No infection was obtained with the cauliflower mosaic virus on *Nicotiana glutinosa* and Turkish tobacco (*16*), whereas the turnip mosaic virus caused necrotic lesions to form on inoculated leaves of both *Nicotiana* species. These viruses can also be further differentiated by comparing their properties.

Recently, Tompkins and Thomas (*18*) have described a mosaic disease of Chinese cabbage in which it was shown that this virus can readily be differentiated from the turnip mosaic and cauliflower mosaic viruses on such hosts as cabbage, Chinese cabbage, cauliflower, and turnip.

It is believed the evidence submitted in the present paper is sufficient to differentiate the turnip mosaic virus from other viruses recently described (*16, 17, 18*).

DISCUSSION

A review of the literature on turnip mosaic (*1, 4, 5, 6, 7, 9, 11, 12, 13, 19*) and rutabaga mosaic (*2, 6, 8, 14, 19*) indicates a lack of agreement on the part of the workers concerned, specific examples of which may be cited. Although it seems to be generally agreed that the principal symptom of the disease on turnip consists of leaf mottling, additional symptoms unlike in any two instances are given by several writers (*1, 2, 4, 5, 7, 9, 12*). All but Gram (*5*) reported infection by mechanical means. Concerning insect transmission, Schultz (*12*) proved the green peach aphid was a vector; Pape (*9*) considered the tarnished plant bug was responsible for natural spread of the disease in Germany; while Hoggan and Johnson (*7*) and Chamberlain (*1*) found that both the green peach and cabbage aphids were efficient vectors. In studies on the rutabaga mosaic virus, Clayton (*2*) stated the green peach aphid gave unsatisfactory results, but transmission occurred by means of the cabbage aphid. Schultz (*12*), Clayton (*2*), and Chamberlain (*1*) reported negative results in tests for seed transmission but no data were given by others. According to Gardner and Kendrick (*4*), the incubation period for artificial infection ranged from 16 to 26 days while Clayton (*2*) records 3 to 5 weeks. Schultz (*12*) reported a period of 12 to 20 days for the green peach aphid.

Relative to host range, Gardner and Kendrick (*4*) and Chamberlain (*1*) were unable to infect radish. Unfortunately, other workers failed to test this plant for susceptibility. Conversely, Gram (*5*) observed infection of radish. Infection of rutabaga was reported only by Gram (*5*), Clayton (*2*), and Chamberlain (*1*); the description of symptoms differs in the latter two instances. There is no close agreement concerning symptoms on such hosts as Brussels sprouts and cauliflower based on the studies of Clayton (*2*) and Chamberlain (*1*). Clayton (*2*) found that cabbage was practically immune, Hoggan and Johnson (*7*) observed chlorotic rings, and Chamberlain (*1*) noticed only a faint mottle on the leaves. The writer (*16*) has previously reported light-green to yellow lesions which become necrotic around the edges with age. Spinach and currant tomato were reported as hosts by Hoggan and Johnson (*7*), but the writer has failed to obtain confirmatory results. To date, data on the properties of a turnip mosaic virus have been published only by Hoggan and Johnson (*7*). They used tobacco as a test host and since the writer used turnip a satisfactory comparison cannot be made.

Inevitably these differences have led to such questions as: Was the same turnip mosaic virus, or the same rutabaga mosaic virus, under the consideration by the different workers; are the turnip mosaic and rutabaga mosaic viruses identical? It is believed satisfactory answers to these questions cannot be given on the basis of available information and that any definite conclusions would not be justified.

SUMMARY

A mosaic disease of turnip, prevalent on Long Island, N. Y., is described.

Turnip mosaic is characterized by coarse vein clearing of the leaves in early stages of infection followed by conspicuous mottling with raised islands, crinkling, and stunting of the plants.

Under greenhouse conditions the turnip mosaic virus was readily transmitted by *Myzus persicae* and *Brevicoryne brassicae*. The virus was also transmitted by mechanical inoculation using carborundum as an abrasive; the incubation period ranged from 13 to 21 days. In longevity tests, the virus was active at the end of 2 days but inactivated after aging for 3 days at 22° C. Its inactivation temperature lies between 60° and 63°. A tolerance to dilution of 1 to 3,000 was established.

The host range includes 18 species of plants representing 12 genera in 6 families; 11 species belong to the family Cruciferae and include cabbage, cauliflower, rutabaga, leaf or Chinese mustard, pe-tsai, annual stock, dames violet, Virginian stock, honesty, and Chinese radish.

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ASSAYS OF RIBOFLAVIN AND THE FILTRATE FACTOR IN CERTAIN MILK PRODUCTS[1]

By T. H. JUKES, *junior poultry husbandman*, and G. A. RICHARDSON, *assistant dairy chemist, California Agricultural Experiment Station*[2]

INTRODUCTION

Two factors in the vitamin G group are known to be needed by chicks.[3] The first of these is riboflavin (flavin, lactoflavin), which is of great importance for growth and hatchability, and the second is the "filtrate factor," which prevents a dermatitis caused by a dietary deficiency in chicks. Dried milk and dried whey supply both of these vitamins (*4*). Milk products are particularly important in poultry rations as sources of riboflavin, since many feeding stuffs, such as the cereal grains, are deficient in this substance. Heiman and Carver (*1*) have recently found that a sample of neutralized dried whey contained between 10 and 20 percent more vitamin G, and a sample of dried sweet-cream buttermilk contained between 90 and 100 percent more vitamin G, than a sample of dried skim milk. The samples were prepared from similar supplies of milk collected during 1 week.

In the present investigation two series of spray-dried milk products were studied. Each series originated from a single batch of whole milk. Each product was biologically assayed for the filtrate factor and for riboflavin by means of chick growth.

PREPARATION OF SPRAY-DRIED MILK PRODUCTS

For the products prepared in November 1935, a mixed composite of 7,419 pounds of milk was obtained from 23 patrons. Approximately 1,600 pounds of this was Guernsey milk; the remainder was Jersey milk. The cows received pasture, rye hay, carrots, beets, and millfeed consisting largely of rolled barley, coconut meal, and beet pulp. The milk was standardized down to 4.5 percent of milk fat (Babcock) by the removal of cream. It was then subdivided into appropriate lots for the preparation of the respective products.

For the products prepared in May 1936, a mixed composite of 5,660 pounds of milk from 19 patrons was used. This milk was obtained from about the same territory as the November milk, although not necessarily from the same patrons. Pasture and some millfeed constituted the main ration of the cows. This milk was standardized down to 4.287 percent of fat (Mojonnier) (4.34 percent Babcock) by the removal of cream. It was then subdivided into lots.

The buttermilk was obtained by churning the cream received the day previous in the same plant, although not necessarily from the same patrons who furnished the milk for the remainder of the products.

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[1] Received for publication September 16, 1937; issued November 1938.

[2] The samples used in this work were obtained through the cooperation of Dr. Paul D. V. Manning and Mr. Henry Pollard of the Western Condensing Co., San Francisco. The Golden State Co., San Francisco, placed their spray-drying plant at Ferndale, Calif., at the disposal of the writers for the preparation of the samples. The analyses of the products were made by Miss M. A. Ashenfelder, analyst, University of California, Davis, Calif. Washed casein was furnished by the California Milk Products Corporation, Gustine, Calif., through the courtesy of J. Chrisman.

[3] Italic numbers in parentheses refer to Literature Cited, p. 609.

All other products in each series were prepared from the one lot of milk.

The whole milk, skim milk, and buttermilk were dried in the usual manner by the Gray-Jensen process. The various casein wheys were obtained by the customary technique. The hydrochloric acid whey was neutralized with soda ash; the sulphuric acid whey, with lime. The lactic acid whey was not neutralized. The rennet whey was the usual Cheddar-cheese whey. Table 1 shows the analyses of the various products.

TABLE 1.—*Chemical analyses of milk and dried-milk products*

Product	Month prepared	Fluid product			Dried product			
		Fat [1]	Total solids	pH	Fat	Total solids	Solids not fat	Ash
		Percent	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*
Whole milk	November	4.5			30.16	94.14	62.94	4.76
	May	4.287	13.12	6.41	32.35	99.01	66.66	4.81
Skim milk	November		10.28			98.32		7.44
	May	.071	9.41	6.44	1.29	97.72	96.43	9.05
Buttermilk	November		9.80			98.32		7.24
	May	.465	9.43	6.36	4.84	98.15	93.31	6.85
Rennet whey (Cheddar)	November		6.65			97.91		6.56
	May	.092	6.27	6.10	1.43	97.62	97.19	6.45
Hydrochloric acid casein whey (neutralized).	November		6.71			97.70		11.56
	May		6.66	6.14	.30	98.79	98.49	11.77
Sulphuric acid casein whey (neutralized).	November		6.92			96.42		14.62
	May		6.54	6.46	.38	97.38	97.00	11.50
Lactic acid casein whey	November		6.62			91.92		10.98
	May		5.95	4.73	.35	97.60	97.25	8.99

[1] Fat was determined by the Mojonnier method, except for the fluid whole milk for November.

BIOLOGICAL ASSAY FOR THE FILTRATE FACTOR

METHOD

The method used to determine the filtrate factor was the one employed in previous investigations (*2*, *6*). Chicks were placed on an ordinary mixed diet at hatching and kept on the diet for 6 to 8 days. They were then placed on a heated diet of natural foodstuffs (*2*, *5*) for 8 to 10 days, after which they were weighed and divided into groups of 10. The test diets were then fed for 12 to 14 days, and the growth response was noted. Each series was controlled by a group on the basal heated diet 80G (*2*) and a group on the positive control diet, which consisted of the heated diet plus 10 percent of rice-bran filtrate (*2*). The filtrate factor content of a sample of dried whey, for example, was computed as follows:

$$\text{Filtrate factor content of whey sample in units per gram} = \frac{\text{Growth on diet supplemented with whey minus growth on basal diet}}{\left\{\begin{matrix}\text{Growth on positive control diet}\\ \text{minus growth on basal diet}\end{matrix}\right\} \times \begin{matrix}\text{Grams of whey in 1}\\ \text{g. of test diet.}\end{matrix}}$$

If, for example, during the assay period the average growth was 59 g on the positive control diet, 4 g on the basal diet, and 33 g on a test diet containing 12 percent of dried whey, the filtrate-factor content of the dried whey would then be computed as

$$\frac{33-4}{(59-4)\ 0.12} = 4.4 \text{ units per gram } (2).$$

The diets were adjusted to keep the nutritive ratio fairly uniform. Table 2 illustrates the composition of the diets in a typical test. The whey adsorbate used was assayed for riboflavin with chicks. From 1.3 to 1.5 percent supplied enough riboflavin for maximal growth between 2 and 4 weeks when fed in conjunction with diet 96.

TABLE 2.—*Composition of diets* [1] *(expressed in parts) used in a typical assay of dried-milk products for the filtrate factor*

Constituent	Composition of diet No. —								
	80G 90	80G 91	80G 92	80G 93	80G 100	80G 96	80G 95	80G 97	80G 98
Yellow corn meal heated at 120° C. for 24 hours	34	46	46	46	46	46	46	56	56
Wheat middlings heated at 120° C. for 24 hours	25	25	25	25	25	25	25	25	25
Commercial casein heated at 120° C. for 24 hours	10	4	10	10	4	10	10	12	12
Sodium chloride	1	1	1	1	1	1	1	1	1
Ground limestone	1	1	1	1	1	1	1	1	1
Steamed bonemeal	1	1	1	1	1	1	1	1	1
Whey adsorbate	2	2	2	2	2	2	2	2	2
Cod-liver oil, medicinal	1	2	2	2	2	2	2	2	2
Dried whole milk	25								
Dried skim milk					18				
Dried buttermilk		18							
Dried rennet whey			12						
Dried hydrochloric acid casein whey				12					
Dried lactic acid casein whey						12			
Sulphuric acid casein whey							12		
Rice-bran filtrate									10

[1] Hexane extract of alfalfa meal, equivalent to 1 percent of alfalfa meal, evaporated on all diets to supply vitamin K.

RESULTS

The results are summarized in table 3, which compares the various products with respect to their filtrate-factor content in units per gram of the spray-dried material. The dried wheys contained a higher concentration of the filtrate factor because this vitamin is not readily adsorbed and remains in solution in whey after the removal of the fat and casein. Table 3 shows that there may be considerable disparity between duplicate assays; but when average values were taken as in table 4 the products prepared in May arranged themselves in the same order of efficacy as those prepared in November. Differences between a product prepared in November and the corresponding product prepared in May were insignificant.

TABLE 3.—*Filtrate factor content of dried milk and whey samples prepared in November 1935 and May 1936 as determined by biological assay with chicks*

NOVEMBER SAMPLES

Diet No.	Supplement (dried product)	Proportion of supplement in diet	Length of assay period	Gain on supplemented diet	Gain on basal diet	Gain on positive control	Filtrate factor per gram of supplement
		Percent	*Days*	*Grams*	*Grams*	*Grams*	*Units*
80E 58	Skim milk	18	13	32	4	59	2.8
80E 59	Whole milk	25	13	36	4	59	2.3
80E 60	Buttermilk	18	13	44	4	59	4.0
80E 61	Rennet whey	12	13	43	4	59	5.9
80E 62	Hydrochloric acid casein whey	12	13	30	4	59	3.9
80E 63	Lactic acid casein whey	12	13	34	4	59	4.5
80E 64	Sulphuric acid casein whey	12	13	35	4	59	4.7
80E 68	Skim milk	12	14	25	8	72	2.2
80E 69	Whole milk	12	14	13	8	72	0.7
80E 70	Buttermilk	12	14	27	8	72	2.5
80E 71	Rennet whey	12	14	41	8	72	4.3
80E 72	Hydrochloric acid casein whey	12	14	28	8	72	2.6
80E 73	Lactic acid casein whey	12	14	30	8	72	2.9
80E 74	Sulphuric acid casein whey	12	14	39	8	72	4.0

MAY SAMPLES

80G 100	Skim milk	18	12	35	6	64	2.8
80G 90	Whole milk	25	12	29	6	64	1.6
80G 91	Buttermilk	18	12	39	6	64	3.2
80G 92	Rennet whey	12	12	41	6	64	5.0
80G 93	Hydrochloric acid casein whey	12	12	26	6	64	2.9
80G 94	Lactic acid casein whey	12	12	40	6	64	4.9
80G 96	Lactic acid casein whey	12	12	38	6	64	4.6
80G 95	Sulphuric acid casein whey	12	12	38	6	64	4.6
80G 110	Skim milk	24	13	25	8	55	1.5
80G 103	Whole milk	33	13	34	8	55	1.7
80G 104	Buttermilk	24	13	46	8	55	3.4
80G 105	Rennet whey	16	13	54	8	55	6.1
80G 106	Hydrochloric acid casein whey	17	13	34	8	55	3.2
80G 108	Lactic acid casein whey	15	13	30	8	55	3.1
80G 107	Sulphuric acid casein whey	17	13	50	8	55	5.3

TABLE 4.—*Average values obtained for filtrate factor content of dried-milk products in units per gram*

[Summarized results of table 3]

Dried-milk product	November 1935 samples	May 1936 samples	Dried-milk product	November 1935 samples	May 1936 samples
	Units	*Units*		*Units*	*Units*
Skim milk	2.5	2.2	Hydrochloric acid casein whey	3.2	3.0
Whole milk	1.5	1.6	Lactic acid casein whey	3.7	4.2
Buttermilk	3.2	3.3	Sulphuric acid casein whey	4.4	5.0
Rennet whey	5.1	5.6			

BIOLOGICAL ASSAY FOR LACTOFLAVIN

METHOD

In an assay for the filtrate factor, the basal diet must contain an excess of riboflavin (*6*). For the same reason, a basal diet for the biological assay of riboflavin must contain an excess of the filtrate factor. Diet 96 (*7*) was used. The treatment of the chicks and the

method of calculating the riboflavin content of the supplements resembled those employed in filtrate-factor assays except, of course, that diet 96 was used instead of diet 80G and the supplement for the positive control diet was whey adsorbate instead of rice-bran filtrate. Table 5 shows the composition of the diets and some of the results obtained. The rice-bran filtrate furnished 20 units of filtrate factor per gram.

RESULTS

Several complete series of riboflavin assays of the two sets of milk products were made. The results are shown in table 6 and summarized in table 7. Although fairly consistent values were obtained for the dried-milk samples, the variation in the results obtained for the whey samples was disappointingly large. At present the discrepancies in the whey assays cannot be explained. Other materials such as alfalfa meal have given a fair degree of reproducibility in repeated assays.

Recent unpublished studies indicate that the "unit of riboflavin" employed in this investigation corresponds to about 2.3 micrograms of synthetic d-riboflavin.

TABLE 5.—*Representative diets used (expressed in parts) and weight gains made in assay of dried-milk and whey products for riboflavin by the method of chick growth*

Constituent	Composition of diet No.—										
	96-86	96-87	96-88	96-89	96-90	96-91	96-92	96-100	96-101	96-102	96-103
Yellow corn meal	30	30	30	30	30	30	30	30	30	30	30
Cornstarch	20	17	22	22	22	22	22	23	23	23	23
Washed bran	10	10	10	10	10	10	10	10	10	10	10
Washed casein	19	19	20	21	21	21	21	22	22	22	22
Rice-bran filtrate, free from lactoflavin	7	7	7	7	7	7	7	7	7	7	7
Salt	1	1	1	1	1	1	1	1	1	1	1
Limestone	1	1	1	1	1	1	1	1	1	1	1
Bonemeal	1	1	1	1	1	1	1	1	1	1	1
Alfalfa hexane extract [1]											
Cod-liver oil	2	2	2	2	2	2	2	2	2	2	2
Soybean oil	3	3	3	3	3	3	3	3	3	3	3
Dried skim milk (November 1935)	6										
Dried whole milk (November 1935)		8.7									
Dried buttermilk (November 1935)			4								
Dried hydrochloric acid casein whey (November 1935)				3.9							
Dried rennet whey (November 1935)					3.9						
Dried sulphuric acid casein whey (November 1935)						4					
Dried lactic acid casein whey (November 1935)							3.9				
Whey adsorbate									1	2	2.5
Gain of chicks in 15-day assay period.....grams	48	48	53	48	39	39	42	18	55	64	65
Gain over basal.....do	30	30	35	30	21	21	25		37	46	47
Riboflavin content of supplement per gram.....units	11	7	19	17	12	12	14		80	(2)	(2)

[1] Equivalent to 1 of alfalfa meal.
[2] Positive control diets.

TABLE 6.—*Riboflavin content of dried milk and whey samples prepared November 1935 and May 1936, as determined by biological assay with chicks*

Diet No.	Supplement (dried product)	Proportion of supplement in diet	When prepared	Period on diet	Gain on supplemented diet	Gain on basal diet	Gain on positive control diet	Riboflavin per gram of supplement
		Percent		*Days*	*Grams*	*Grams*	*Grams*	*Units*
93 2	Skim milk	5	November	20	49	22	71	11
93 3	Whole milk	7	do	20	51	22	71	8
93 4	Rennet whey	3.2	do	20	46	22	71	15
93 5	Hydrochloric acid casein whey	3.3	do	20	37	22	71	9
93 6	Lactic acid casein whey	3.2	do	20	35	22	71	8
93 7	Sulphuric acid casein whey	3.4	do	20	39	22	71	10
93 8	Buttermilk	5	do	20	62	22	71	16
96 16	Skim milk	5	do	14	28	9	53	9
96 17	do	5	May	14	34	9	53	11
96 19	Buttermilk	5	do	14	41	9	53	15
96 23	Skim milk	8	November	15	42	12	58	8
96 31	Lactic acid casein whey	5.1	May	15	33	12	58	9
96 32	Rennet whey	5.3	do	15	31	12	58	8
96 86	Skim milk	6	November	15	48	18	64	11
96 87	Whole milk	8.7	do	15	48	18	64	7
96 88	Buttermilk	4	do	15	53	18	64	19
96 89	Hydrochloric acid casein whey	3.9	do	15	48	18	64	17
96 90	Rennet whey	3.9	do	15	39	18	64	12
96 91	Sulphuric acid casein whey	4	do	15	39	18	64	12
96 92	Lactic acid casein whey	3.9	do	15	43	18	64	14
96 93	Skim milk	6	May	15	47	18	64	11
96 96	Hydrochloric acid casein whey	4.3	do	15	50	18	64	16
96 97	Rennet whey	4	do	15	35	18	64	9
96 98	Sulphuric acid casein whey	4.2	do	15	32	18	64	7
96 99	Lactic acid casein whey	3.8	do	15	36	18	64	10
96 116	Whole milk	8.3	do	14	62	18	75	9
96 117	Buttermilk	4	do	14	61	18	75	19
96 124	Sulphuric acid casein whey	4	November	14	47	9	70	16
96 125	Rennet whey	4	May	14	47	9	70	16
96 126	Skim milk	6	November	14	61	9	70	14
96 127	Hydrochloric acid casein whey	4	do	14	42	9	70	14
96 128	Rennet whey	4	do	14	57	9	70	19
96 129	Lactic acid casein whey	4	do	14	47	9	70	16
96 132	Skim milk	6	May	14	50	9	70	11
96 133	Hydrochloric acid casein whey	4	do	14	45	9	70	15
96 134	Sulphuric acid casein whey	4	do	14	48	9	70	16
96 135	Lactic acid casein whey	4	do	14	47	9	70	16

TABLE 7.—*Average values obtained for riboflavin content of dried-milk products in units per gram*

[Summarized results of table 6]

Dried-milk product	November 1935 samples					May 1936 samples		
	Units	*Units*	*Units*	*Units*	*Units*	*Units*	*Units*	*Units*
Skim milk	11	9	8	11	14	11	11	11
Whole milk	8	7	.....	.....	.....	9	.....	.....
Buttermilk	16	19	.....	.....	.....	15	19	.....
Hydrochloric acid casein whey	9	17	14	.....	.....	16	15	.....
Rennet whey	15	12	19	.....	.....	8	9	16
Sulphuric acid casein whey	10	12	16	.....	.....	7	16	.....
Lactic acid casein whey	8	14	16	.....	.....	9	10	16

SUMMARY

A series of spray-dried milk products was prepared from a batch of milk in November 1935, and a similar series was prepared at the same place in May 1936. Each series consisted of whole milk, skim milk, buttermilk, rennet whey, hydrochloric acid casein whey, sulphuric acid casein whey, and lactic acid casein whey. Each series of samples was submitted to biological assay for the filtrate factor with chicks on a basal diet of heated natural foodstuffs. Any possible differences between the November and the corresponding May samples were not large enough to be detected by the method of assay. The descending order of potency for the November series was as follows: Rennet whey, sulphuric acid casein whey, lactic acid casein whey, hydrochloric acid casein whey and buttermilk, skim milk, whole milk. The order was the same for the May series except that buttermilk was slightly superior to hydrochloric acid casein whey. In rough figures, dried whey of various types furnished from 3 to 6 units of filtrate factor per gram; dried buttermilk, 3 to 4; dried skim milk, 2 to 3; and dried whole milk, 1 to 2.

Dried milk and dried whey are not concentrated sources of the filtrate factor, but it was possible to assay spray-dried milks and wheys with fairly good agreement between duplicate assays.

Milk products supply from two to five times as much riboflavin as the filtrate factor in terms of similarly computed chick units. Milk is valuable, therefore, in the chick ration as a source of riboflavin, since grains and grain byproducts present an opposite situation—that is, they tend to be very poor sources of riboflavin and to supply moderate amounts of the filtrate factor. Recent unpublished studies indicate that 1 chick unit corresponds to about 2.3 micrograms of synthetic d-riboflavin in terms of the methods employed in this investigation.

No difference within the limits of accuracy of the biological assay method used was found between November and May samples of milk products from the same locality.

Buttermilk was distinctly richer than skim milk in riboflavin.

Inconsistencies in the results made it impossible to say whether there were appreciable differences in the riboflavin content of whey samples prepared by different methods from the same batch of milk. As table 7 shows, fairly consistent results were obtained in repeated assays of skim milk, whole milk, and buttermilk; but the results with the various whey samples were irregular. At present the discrepancies in the whey assays cannot be explained. Other materials, such as alfalfa meal, have given a fair degree of reproducibility in repeated assays.

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RELATION OF SOIL TEMPERATURE TO CHLOROSIS OF GARDENIA[1]

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INTRODUCTION

Normal leaves of gardenia are glossy and dark green in color. Immature leaves may be a lighter green, but they are glossy and quite green even as they open from the bud. During the winters of 1934–35 and 1935–36 a chlorotic condition of the leaves of gardenia growing under glass caused serious concern to Massachusetts growers. The malady in its early stages is characterized by a gradual decrease of green in the interveinal areas of the leaves. This decrease of green continues until a straw-yellow color has developed. The vein areas are the last to lose the green color. This type of chlorosis is first noted near the tips of shoots, i. e., newly developed and rapidly growing branches, and is followed by a similar chlorotic development in the leaves of the longer terminal branches. In later stages growth slows down and new leaves, as they appear, are relatively small and lack green color. Frequently, brown areas develop at the tip of the leaf and also in the margin.

Chlorosis is the visible effect of some cause, but it is by no means a criterion to establish a definite cause. Among the causes suggested for the chlorosis in question were the following: Deficiency in iron; soil reaction; unbalanced fertilizer ration; high concentration of calcium and sodium ions; nitrogen deficiency; lack of light; and mosaic diseases. A careful study of the environment and treatment of plants, some healthy and some chlorotic, indicated that none of these factors was involved. However, there did develop some evidence that a temperature factor was involved. Further investigation revealed that this temperature factor was localized in the soil.

METHODS

A preliminary test of the theory that soil temperature was the controllable factor in the chlorosis of gardenia was made by placing chlorotic potted gardenia [2] plants on a shelf over steam pipes. Check plants, not quite so chlorotic, were kept on a bench that did not have bottom heat. The test was started February 14 and was continued for 22 days. Records of air temperature about the two sets of plants showed the air temperature to be substantially the same. Soil-temperature records were taken daily at 9 a. m.

At about the twelfth day, the plants over the steam pipes showed a noticeable change in the tip leaves. The yellow color was disappearing as the healthy green color returned. These were young leaves and

[1] Received for publication February 11, 1938; issued November 1938. Published as Contribution No. 301 of the Massachusetts Agricultural Experiment Station.

[2] *Gardenia veitchii* was used in all the tests reported.

they developed well to their normal size. Leaves below the tip leaves were too old to expand to normal size, but they did regain their normal green color, which started from the base of the midrib and slowly expanded outwardly from the midrib. In general, the return of the green color was from the top of the plant downward and from the base of the leaf outward.

The check plants, less chlorotic at the start of the test, became exceedingly chlorotic. The newer leaves lacked green color as they unfolded and they developed to only a small fraction of their normal size. The record of the soil temperatures, shown in table 1, indicates the wide difference in this factor during the duration of the test.

TABLE 1.—*Soil temperature records of, and the temperature difference between, plants on benches with and without bottom heat*

Date	Bottom heat	Without bottom heat	Difference	Date	Bottom heat	Without bottom heat	Difference
	°C.	°C.	°C.		°C.	°C.	°C.
Feb. 15	23	18	5	Feb. 29	25	14	11
Feb. 16	26	17	9	Mar. 1	31	17	14
Feb. 17	28	18	10	Mar. 2	25	13	12
Feb. 18	23	15	8	Mar. 3	29	18	11
Feb. 19	22	11	11	Mar. 4	28	17	11
Feb. 20	22	10	12	Mar. 5	26	17	9
Feb. 21	23	11	12	Mar. 6	22	13	9
Feb. 22	31	17	14	Mar. 7	29	19	10
Feb. 23	23	14	9				
Feb. 24	24	14	10	Maximum	31	20	14
Feb. 25	30	18	12	Minimum	21	10	5
Feb. 26	30	20	10				
Feb. 27	27	17	10	Average	25.8	15.5	10.3
Feb. 28	21	13	8				

The recognition of soil temperature as a causal factor made it possible to check conditions in a greenhouse where chlorosis was well established. There were, however, plants or groups of plants that were healthy. Plants in containers near or over steam pipes had healthy dark-green leaves. Plants in benches, except at the ends of the house, were generally chlorotic. At one end of the house, steam entered the radiating system and at the other end, with extra piping and traps, steam was condensed and the water stored before being returned to the boiler. Soil-temperature readings in these healthy areas were markedly higher than those in the chlorotic areas. The lowest soil temperature in this particular house was in a bench about which steam seldom passed. It was in this bench that chlorosis always appeared first and became most severe.

The demonstration that this particular type of chlorosis could be obviated by raising the soil temperature, and the fact that chlorosis was prevalent at all low soil temperatures and absent at high soil temperatures, clearly indicated that somewhere within a wide range of soil temperatures there would exist a narrow band of temperatures below which chlorosis would be induced and above which it would not appear.

Experiments were, consequently, carried out in a constant soil-temperature apparatus [3] where it is possible to obtain and maintain a wide or narrow range of soil temperatures (fig. 1). The experiments

[3] FULLER, JAMES E., and JONES, LINUS H. THE INFLUENCE OF TEMPERATURE ON THE NITRATE CONTENT OF SOIL IN THE PRESENCE OF DECOMPOSING CELLULOSE. Soil Sci. 34: 337–350, illus. 1932.

were definitely planned not only to study the effect of soil temperature as the days grew shorter, but also to make a similar study as the daylight period lengthened. It was further planned that the influence

FIGURE 1.—Constant soil-temperature apparatus in which gardenias were exposed to a range of soil temperatures.

by the light factor in the apparatus must be overcome by inducing chlorosis by soil temperature at any and all available locations in the apparatus. The influence of previous exposure of the plant roots to

soil temperature was considered by establishing the plants at known soil temperatures before they were subjected to different soil temperatures.

EXPERIMENTAL RESULTS

EXPERIMENT 1, MARCH 25–JUNE 16

Hard plants from 3½-inch pots were shifted to galvanized-iron containers. There were two plants in each container and four containers at each temperature in the apparatus. The range of temperatures was from 8° to 32° C., the increment of increase being at 2° steps. The air temperature was maintained at about 21° by a thermostat control. However, for 14 days before the temperature adjustment was made, all plants were allowed to establish themselves at a soil temperature of 24°. The soil used was 4 parts good compost soil and 1 part peat.

Effect of Soil Temperature on the Plant

The first effect of soil temperature was noted at the two lower temperatures, 8° and 10° C. The plants wilted immediately as the temperature adjustment was attained. They recovered their turgidity at night, and maintained it in cloudy weather; but for 2 weeks they wilted in the sunshine, the tendency naturally decreasing as the period lengthened. Evidently root activity was depressed by the low temperature, and the roots could not absorb enough water to replace that lost by transpiration in periods of sunshine. This type of wilting, due to a lowered root temperature, has also been noted by Arndt,[4] who observed it in cotton plants grown in solution and in soil cultures.

The second effect was also noted at the two lowest temperatures where there occurred a rapid senescence of the oldest leaves, i. e., those nearest the soil surface. These leaves had become orange in color within 10 days and either dropped or slowly turned to the bronze brown of a dead leaf while remaining attached to the plant. This effect always developed in succeeding experiments and at the same soil temperature of 10° C. or less. At soil temperatures of 12° and above, there was no sign of rapid senescence, although now and then a leaf did turn orange, a perfectly normal occurrence. Undoubtedly the low soil temperature was the causal agent and not the shock of transplanting.

The third effect was the development of interveinal chlorosis. The transition from a healthy green to the early expression of yellow green in chlorosis was hardly noticeable, especially on bright days. It was best observed in cloudy weather. At the end of 40 days, it was apparent that interveinal chlorosis was present in all plants at soil temperatures of 18° C. and below. After 80 days there were a few leaves on plants at the soil temperature of 20° that showed interveinal chlorosis, but it was very slight. All chlorotic leaves at 18° and below became more chlorotic as the test progressed, and the degree of yellowing was directly associated with each drop in soil temperature.

Chemical Analysis of Plant Parts

The leaves and terminal twigs of healthy and chlorotic plants were harvested at the end of 83 days, dried for 48 hours at a temperature of

[4] Arndt, Charles H. Water absorption in the cotton plant as affected by soil and water temperatures. Plant Physiol. 12: 703–720. 1937.

73° C., and chemically analyzed, with the object of determining whether an essential element was lacking. The results of the analysis are shown in table 2.[5]

TABLE 2.—*Chemical analysis of leaves and twigs from healthy and chlorotic gardenia plants*

Constituent	Leaves		Terminal twigs	
	Chlorotic; 20° C. and lower	Healthy; 24° C. and higher	20° C. and lower	24° C. and higher
	Percent	*Percent*	*Percent*	*Percent*
Ash	8.82	6.93	8.09	8.86
Organic matter	91.18	93.07	91.31	91.14
	100.00	100.00	100.00	100.00
Phosphoric acid (P_2O_5)	.46	.46	.60	.99
Nitrogen	3.36	2.84	2.81	2.33
Chlorine (as chlorides)	.16	.16	.10	.10
Sulphuric acid (SO_3) as sulphates	.55	.27	.27	.27
Potassium oxide (K_2O)	2.59	1.50	1.47	1.73
Sodium oxide (Na_2O)	.98	.43	.64	.44
Iron and aluminum oxides (Fe_2O_3, Al_2O_3)	.09	.11	.09	.09
Calcium oxide (CaO)	1.14	1.06	2.87	2.37
Magnesium oxide (MgO)	.54	.60	.78	.75
Manganese (Mn)	.008	.006	.005	.0024
Insoluble matter	.29	.47	.13	.19

There do not appear in the tabulation of the chemical analysis sufficient differences to warrant the interpretation that an essential element is lacking in the chlorotic plants. As a matter of fact, some of the elements are more concentrated in the chlorotic plants, a condition commonly found in diseased or unhealthy plants. While it is possible that at the lower soil temperatures, absorption from the soil by the plant or translocation within the plant may have been depressed, the fact remains that the plants were returned to a normal development by simply raising the soil temperature, which indicated the presence, in adequate quantity, of the necessary chemical constituents.

EFFECT OF SOIL TEMPERATURE ON SIZE OF LEAF

In due time it was evident that the effect of soil temperature expressed itself not only in the degree of chlorosis, but also in the degree of growth and size of leaf. It is of particular interest to note that the size of the leaf was not at all influenced by the air temperature, which was maintained fairly constant the entire length of the apparatus while the soil temperature was the variable. This variable of soil temperature was related to leaf size as measured by length of leaves.

With the limited number of plants it was possible to choose for measurement 10 leaves at each temperature. These leaves were all developed and matured after the experiment was started. The measurements were made 50 days after the temperatures were altered. The averages of the measurements at each soil temperature were taken and graphed as shown in figure 2. These measurements show that the length of the leaf was increased by a rise in soil temperature. No measurable growth took place at the lowest temperature, 8° C., although the plant attempted to form new leaves. These soon withered

[5] This analysis was made by Prof. H. D. Haskins of the Massachusetts Agricultural Experiment Station.

and died in the early effort of expanding. Figure 2 shows a close correlation between size of leaf and chlorosis, indicating that the root medium must supply not only mineral nutrients and water for the plant, but also a temperature environment at which the above-ground portions may carry on their normal functions. In figure 2 the leaves at soil temperature 22° are blocked off from both chlorotic and healthy areas because, though their length was that of healthy leaves, there were slight traces of chlorosis.

EXPERIMENT 2, SEPTEMBER 22–MAY 24

Experiment 2 was planned in two parts: Part 1, to repeat experiment 1 as the days were growing shorter (the days were growing longer in experiment 1); part 2, to shift the temperatures at a suitable time to determine whether the position of the plants in the apparatus

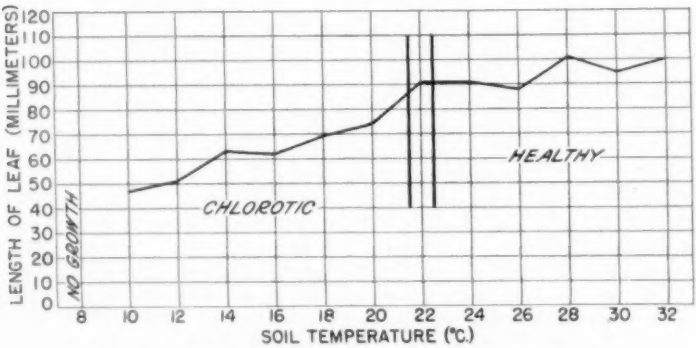

FIGURE 2.—Effect of soil temperature on length of leaves.

was in any way connected with the physiological disturbances as noted in the first experiment.

The same soil temperatures were used except the one at 8° C., which in experiment 1 proved too low for growth. The plants used in this experiment were in a soft condition, vigorous and rapidly growing, in 5-inch clay pots. They were properly acclimated in the apparatus and the range of soil temperatures adjusted.

The development of chlorosis was first evident, after 27 days, in the plants at the lowest soil temperature, 10° C. At the end of 60 days there was the same general distribution of chlorosis as in experiment 1. All new leaves at soil temperatures of 10° and 12° were pale yellow. At 14°, 16°, and 18° the chlorosis was characterized by interveinal yellowing, which was less at each rise in soil temperature. All plants at 22° and above were a normal healthy green, but at 20°, the same uncertain point as in experiment 1, the color of the foliage was normal except on newly formed basal shoots. Such shoots emerge either from below the soil line or just above it. It is possible that the shoots themselves had not developed sufficient tissue to insulate them from the effects of a cool temperature emanating from the soil. Chroboczek [6] has demonstrated that lowered temperatures

[6] CHROBOCZEK, EMIL. A STUDY OF SOME ECOLOGICAL FACTORS INFLUENCING SEED-STALK DEVELOPMENT IN BEETS (BETA VULGARIS L.). N. Y. (Cornell) Agr. Expt. Sta. Mem. 154, 84 pp., illus. 1934.

about the base of a growing point alter the physiological function of the resulting growth.

Twelve weeks (September 22–December 15) after experiment 2 was started and when no further chlorotic effects could be expected, the temperatures, not the plants, were shifted to make sure that the position of the plants in the apparatus was not the determining factor of chlorosis. The plan of the shift and the condition of the plants at the time of shift and at the end of the experiment are shown in figure 3.

The only immediate effect observed from this sudden shift in temperature of the soil was the wilting, at soil temperatures of 10° and 14° C., of the plants which a few hours previously were at soil temperatures of 32° and 28°, respectively. Wilting occurred for a few days, but the plants eventually recovered their turgidity.

Distribution of Temperatures in Apparatus														
(West)							(North)	(East)						24 weeks
10° C.	12° C.	14° C.	16° C.	18° C.	20° C.		22 °C.	24° C.	26° C.	28° C.	30° C.	32° C.		
Condition at end of 12 weeks	Chlorotic							Green						
Plants unchanged; temperatures shifted.														
	32° C.	12° C.	28° C.	16° C.	24° C.	22° C.	20° C.	18° C.	26° C.	14° C.	30° C.	10° C.		
Condition at end of second 12 weeks	Green	Chlorotic (very)	Green	Chlorotic (very)	Green	Green	Light green	Chlorotic (medium)	Green	Chlorotic (medium)	Green	Dark green		

Figure 3.—Diagram to illustrate the effect of soil temperature on chlorosis of gardenia plants regardless of their position in the soil-temperature apparatus.

The plants which had been subjected to low temperatures for 12 weeks (soil temperatures of 10° and 14° C.) were very chlorotic. After they were subjected to higher temperatures (soil temperatures of 32° and 28°), they began to show signs of a return to green color at the thirteenth day. At the end of 56 days, plants which had been green at fairly high soil temperatures (28° and 24°) showed interveinal chlorosis at the changed temperatures of 14° and 18°. The new leaves of these plants were developing with a yellow color instead of the normal green.

Chlorosis did not develop at the lowest soil temperature, 10° C. This exception by no means distorts the previous findings that a low soil temperature will induce chlorosis. This particular group of plants had been growing for 12 weeks at the very high soil temperature of 32°. Under these conditions of excellent root and above-ground environments, the plants were enabled to take advantage of all synthetic processes more intensely than any other group of plants in the experiment. When the temperature of the soil was dropped to 10°, all vegetative growth ceased and the leaves (there were no new leaves

produced after the drop in temperature), instead of becoming chlorotic, became a dark green. The immense supply of food material built up in the 12-week period of high soil temperature was not used in new growth after the temperature was dropped, but evidently was influential in altering the vegetative habit of development to a reproductive habit, for the plants produced a tremendous set of buds, practically every terminal bud developing into a flower bud. There was an average of 34 flower buds per plant, and most of them developed into flowers. The low temperature did not create bud drop. The same alteration from a rapid vegetative growth to reproductive development occurred in the plants which had been growing 12 weeks at 28° and were dropped to 14°, although slight vegetative growth did take place and chlorosis appeared. To a much less extent, the development of flower buds was apparent in the plants originally at the soil temperature of 24° and dropped to 18°. There exists a definite consistency in the relation of high soil temperatures being dropped to fairly low soil temperatures and bud formation. Thus the plants grown at 32°, 28°, and 24° and dropped to 10°, 14°, and 18° respectively (soil temperatures of 30° and 26° were not changed) all produced buds, the number of which was seemingly tied up with the severity of shock to the plant as measured in degrees of temperature dropped. (As this study is confined to chlorosis, the subjects of bud set and bud drop, both little understood, will not be discussed further in this paper.)

In general, the results in experiment 2 were similar to those in experiment 1. They show that soil temperature is the major influence in inducing chlorosis or in causing its disappearance, regardless of time of year. By shifting the position of the temperatures in the apparatus, it was shown definitely that no experimental error due to a light or air environment factor had escaped attention.

It is important to note that those plants that had been at the highest soil temperatures in the first part of experiment 2 developed chlorotic leaves but slowly when the temperature was dropped to a medium level in the second half of the experiment. The results from the second half of experiment 2, while following the trends of the first part, and also the trends of experiment 1, show a delay in developing a chlorotic response, which clearly is related to the soil temperatures and length of time exposures to these soil temperatures before the temperature was dropped. This delay in the onset of chlorosis is also noted in greenhouses, where it has been observed that newly set plants become chlorotic much earlier than plants that have been benched for a year.

EXPERIMENT 3, JUNE 16–NOVEMBER 1

Since, in experiment 2, it was shown that the rate of onset of chlorosis was related to previous soil temperatures and length of exposure to them, it was planned that all sets of plants should be grown over a considerable period at the high soil temperature of 32° C. before being subjected to the range of soil temperatures. Young plants from 2½-inch pots, two in each container, were grown for 90 days at this high soil temperature. Soil temperatures were then adjusted with the increment of increase at 3° instead of 2° in order to make more efficient use of the refrigerating system. However, this increase in the increment did not interfere seriously in making

comparisons of results in experiment 3 with results in experiments 1 and 2.

At the end of 30 days, chlorosis was most intense at the soil temperature of 13° and less intense at 16° C. No chlorosis was apparent at soil temperatures of 19° and above. At the end of 45 days, chlorosis had increased at soil temperatures of 13° and 16°, with a slight indication at 19° and a trace at 22°.

Figure 1 shows the plants in the constant soil-temperature apparatus, and figure 4 shows the chlorotic plant (13°), the healthy plant (32°), and the very dark-green plant (10°). As in experiment 2, the plants at the soil temperature of 10° C. developed no symptoms of chlorosis, but turned a darker green.

FIGURE 4.—Chlorotic gardenia plant, 13° C. (*B*); healthy plant, 32° (*C*); dark-green, nongrowing plant, 10° (*A*).

In experiment 3 a definite attempt was made to corelate the changes in leaf color with growth-vegetative activity. At the time the temperatures were adjusted to the desired range, india-ink markings were placed upon plant stems at all soil temperatures. Enough of these markings were made so that 10 could be chosen as representing typical growth at each temperature. The measurements of increase in length of stem were made at the 45-day period. The averages of these measurements are recorded in table 3, along with the color response as affected by soil temperature. It will be observed that there was no measurable growth at the lowest soil temperature, 10° C. Otherwise, there seems to exist a relation between increase of elongation of stems and the lessening of chlorosis. Although a correlation may exist, and both are traceable to soil temperature as the causal agent, it is difficult to prove that chlorosis is due to a retardation of growth or vice versa. There was no growth at the soil temperature of 10°, neither was there any chlorosis.

TABLE 3.—*Effect of soil temperature on distribution of chlorosis and rate of growth from September 13 to October 27*

Soil temperature (° C.)	Growth	Extent of chlorosis	Soil temperature (° C.)	Growth	Extent of chlorosis
	Centimeters			*Centimeters*	
10	0.0	Dark green.	22	11.6	Trace of chlorosis.
13	4.8	Very chlorotic.	25	12.3	Green.
16	7.7	Chlorotic.	32	13.9	Do.
19	10.5	Slightly chlorotic.			

DISCUSSION

The fact that soil temperature not only induces and controls chlorosis of gardenia, but also affects the above-ground parts in size of leaf, rate of growth, and alteration of the vegetative and reproductive phases, and even produces wilting when lowered to 10° C. or less, emphasizes an important factor for consideration in the science of ecology. While it is customary to consider the effects of temperature on the basis of air-temperature readings, and while there is undoubtedly a correlation of plant response with air temperature, nevertheless, as these experiments show, the correlation with soil temperature may be even closer.

In field and forest no particular control can be exercised on adjustments of soil temperature. However, it might prove profitable to study the various responses that are traceable to soil temperatures.

Under greenhouse conditions, where some degree of control can be exercised, it becomes apparent that the practice of controlling air temperature may have some virtue, but it seems possible that plant response can be guided better by an exact consideration of soil temperature. While sunshine and clouds may alter the air temperature quickly, neither has an immediate effect on soil temperature, which lags several hours in attaining its maximum and minimum in relation to the same extremes of air temperature. This lag is not consistent or easily predictable, since the amount of moisture in the soil is not a constant and may vary considerably from immediately after watering until the watering is repeated. The amount of water applied and its temperature are also factors which may lower the soil temperature only slightly in summer and early autumn, but will lower it considerably in midwinter and early spring. Heating the water to be applied is sometimes practiced, but no recommendations have heretofore been made that soil-temperature readings be made in connection with watering or other greenhouse practices. Depth of soil is also important and one would expect a slower alteration of soil temperature in ground beds than in raised benches.

While the tests discussed in this paper have dealt with *Gardenia veitchii*, it has been observed that the varieties with larger leaves and larger flowers are considerably more susceptible to the influence of soil temperature on chlorosis. It has been observed also that plants benched in August develop chlorosis more quickly than those benched the previous August. Age and length of exposure to high summer temperatures are factors which condition the gardenia plant to resist the early soil-temperature influence on inducing chlorosis.

SUMMARY AND CONCLUSIONS

The discovery that a high soil temperature would dissipate the chlorosis of *Gardenia veitchii* suggested the testing of the effects of soil temperature on this plant. The work was conducted in a constant soil-temperature apparatus capable of supplying a range of soil temperatures at any time of the year. The work was repeated sufficiently to warrant the following statements.

Chlorosis was induced at a soil temperature of 18° C. and less, and developed more intensely directly as the soil temperature was lower.

At soil temperatures of 20° and 22° C. there were traces of chlorosis, but it did not increase in intensity as time went on.

A chemical analysis of leaves and terminal twigs from healthy and chlorotic plants did not indicate a deficiency in nutrient elements. It did show, in the case of leaves, analytical results characteristic of unhealthy plants.

Change in the length of the day did not affect the onset of chlorosis.

The time required to induce chlorosis varied with the condition of the plants when the temperatures were lowered. Hard plants growing at medium temperatures developed chlorosis more slowly than soft plants grown at high temperatures.

The position of the plants in the constant soil-temperature apparatus (daylight and possibly air-temperature factors) had no influence on the induction or dissipation of chlorosis.

A sharp rise in soil temperature maintained for 13 days was sufficient to initiate the gradual return of a healthy green color.

There was an inverse correlation between the rate of growth and the intensity of the development of chlorosis.

Soil temperature is a factor affecting the size of the leaf, the length of the leaf increasing as the temperature is raised.

At the lower soil temperatures, 8° and 10° C., the plants become chlorotic if they have been growing slowly. If they have been growing rapidly at a high temperature, they do not become chlorotic when the temperature is lowered, but develop a very dark green color and cease to grow at all.

Plants growing at a fairly high soil temperature wilt when the temperature is dropped suddenly to 8° and 10° C., but eventually recover their turgidity.

An effect of lowering the soil temperature to 10° C. or less is the rapid senescence of the oldest leaves.

Plants that have been at a high soil temperature and are subjected to a low temperature grow little, but set buds. On the other hand, plants that have been at a low temperature and are subjected to a high temperature grow vigorously, but set no buds. It is evident that soil temperature is a factor that may alter the physiological phases of vegetative and reproductive development.

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RESISTANCE IN THE RED RASPBERRY TO THE MOSAIC VECTOR AMPHOROPHORA RUBI KALT.[1]

By GLENN A. HUBER, *plant pathologist, Western Washington Experiment Station* and C. D. SCHWARTZE, *assistant horticulturist, Washington Agricultural Experiment Station and horticulturist Western Washington Experiment Station*[2]

INTRODUCTION

The presence of red raspberry mosaic[3] in Washington has been discussed by Jones and Baur (*3*).[4] This disease is gradually spreading to new localities, and is of major importance in western Washington because of the high susceptibility of the principal commercial variety, Cuthbert, to infection.

Rankin (*5*), in New York, observed that certain red raspberry varieties, such as Herbert and Latham, escaped infection to a greater extent than others. Slate and Rankin (*7*) state that the reason for the slower spread in one variety than in another is not known but "reflects some varietal character in relation to aphid feeding that causes it to escape infection even when aphids arrive on it from diseased plants." Winter (*8*), in Minnesota, found that the insect vectors of mosaic showed a decided preference for certain varieties. He reported that the Herbert variety exhibited marked resistance to *Amphorophora rubi* Kalt., whereas Latham and King were susceptible. Field counts made in Michigan by Bennett (*1*) showed high aphid populations on Latham, King, St. Regis, and the wild red raspberry, but smaller populations on Cuthbert. Cooley (*2*), in New York, observed a widely fluctuating population of *A. rubi* on cultivated raspberries, in contrast to the steady population on most of the wild red raspberry stocks. He states: "This is no doubt a result of the fact that the wild red raspberries are usually growing in protected and shaded locations where the effects of high winds, driving rains, and summer heat are somewhat abated."

It is generally accepted that certain species of red raspberry aphids are the infective agents in mosaic transmission. In western Washington, *Amphorophora rubi*,[5] (*4*) is the most prevalent and widely distributed of the raspberry aphids and has been shown repeatedly to transmit mosaic. Its active nature and abundance throughout the summer months in contrast to other species that are found occasionally indicate that it is the principal vector of mosaic in red raspberry varieties in this section.

While studying mosaic symptoms and the spread of the disease on various red raspberry varieties in experimental plots and commercial

[1] Received for publication March 19, 1938; issued November 1938. Published as Scientific Paper No. 387, College of Agriculture and Experiment Station, State College of Washington. A brief paper including a part of the data herein reported has been published (*6*).

[2] The writers express their appreciation to A. J. Hanson, entomologist, Western Washington Experiment Station, for suggestions received during the progress of the investigation.

[3] Reference is made in this paper only to the virus disease known as "green" or "severe" red raspberry mosaic.

[4] Italic numbers in parentheses refer to Literature Cited, p. 632.

[5] The writers are indebted to Dr. G. F. Knowlton, associate entomologist, Utah Agricultural Experiment Station, for the identification of this species.

plantings, the writers observed that *Amphorophora rubi* was more abundant on some varieties than on others and was absent from the variety Lloyd George. This indication of resistance to *A. rubi* in the Lloyd George variety appeared to have special significance because of the superiority of this variety in breeding for fruit quality. Examination of young hybrids resulting from crossing Cuthbert, Lloyd George, and other named varieties revealed that some harbored large numbers of aphids, others small numbers, and a few none at all, suggesting that resistance to *A. rubi* may be transmitted in hybridization.

Following these preliminary observations, a more thorough investigation of aphid resistance in named varieties and hybrids was undertaken by means of both greenhouse experiments and field observations. The results obtained from 1935 to 1937, inclusive, are presented in this paper.

MATERIALS AND METHODS

The named varieties of red raspberry (*Rubus strigosus* Michx.) used in greenhouse experiments were from the Western Washington Experiment Station experimental plantings, namely, the variety trial plots maintained for breeding purposes, an isolated plot for foreign introductions, and an isolated planting maintained for raspberry disease investigations. Field studies were made in the same plots and additional data were obtained from commercial plantings throughout the Puyallup Valley. All of the plots from which data were obtained had been established for a sufficient length of time to insure aphid infestation.

The investigation of aphid resistance in hybrid raspberries utilized materials previously developed in breeding for winter resistance. For greenhouse studies, young vigorous "sucker" plants, obtained from each hybrid in the field, were transferred to 5-inch pots. In field studies with hybrids, observations were made in duplicate "increase" plots in which each hybrid selection was represented by 6 to 50 plants. These plantings had been established 2 or more years before the aphid counts were made.

In the greenhouse, usually five plants of each variety or hybrid were tested. All plants were tested for aphid resistance either by placing a single potted plant in a voile cloth cage and infesting the cane tips with aphids, or by confining the aphids to the tips of succulent canes in ventilated cellophane bags (fig. 1). The aphids, *Amphorophora rubi*, used in the 1935 experiments were collected from station plots. Those used in 1936 and 1937 experiments were reared in the greenhouse on Cuthbert plants confined in voile cloth cages (fig. 2). The original colony was established from a single apterous stem mother. Transfers were made from one plant to another with small camel's-hair brushes.

In the field, in order to obtain a comparative count of the aphid populations on varieties and hybrids, 10 canes of each, selected at random throughout the plantings, were examined. Because of varying lengths of canes and because the aphids were found more commonly on the upper leaves and tips of the canes, the upper 10 leaves, in addition to the unfolding leaves and cane tips, were examined and the aphids counted.

FIGURE 1.—Ventilated cellophane bags in which aphids were confined to tips of canes of red raspberry varieties and seedlings.

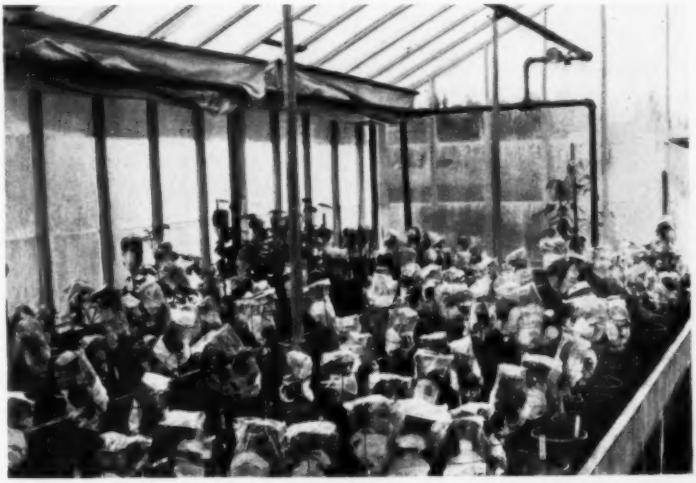

FIGURE 2.—Voile cloth cages in which aphids were reared for the greenhouse tests.

RESISTANCE IN VARIETIES

GREENHOUSE EXPERIMENTS

Preliminary observations on red raspberry varieties in various plantings showed such marked differences in the number of aphids present that two experiments were conducted in the greenhouse in 1935 to determine the behavior of *Amphorophora rubi* on several varieties under controlled conditions.

In the first experiments, young succulent canes of 11 varieties were cut in the field, immediately placed in water, and taken to the greenhouse. Here they were transferred to Erlenmeyer flasks filled with water and so arranged that each flask contained one cane of each of two varieties. All possible varietal combinations were made. The canes in each flask were fastened together at the base and the leaves were allowed to intermingle. All aphids were removed from the canes and 10 aphids, second and third instars, were placed on the tip leaves of each cane. The aphids were collected in the field from varieties different from those on which they were placed. The water in the flasks was changed twice daily, and the temperature in the greenhouse was maintained at 70° to 75° F.

After 7 days the aphids on each cane were counted. Each leaf was carefully removed when the count was made so as to distrub the aphids as little as possible, thus minimizing movement from one variety to another during the process of counting. The results of the experiment are given in table 1.

On the varieties Chief, Cuthbert, King, Latham, Marlboro, Newman, and Viking the aphid population increased abundantly, while on Antwerp, Herbert, Lloyd George, and Newburgh it increased only slightly or not at all. Paired varieties of the former group show in general a marked increase of *Amphorophora rubi* on both, while in paired varieties of the latter group there was a marked decline in numbers of aphids with one exception, the Newburgh-Herbert combination. When varieties of each group were paired the number of aphids increased on varieties of the former group and decreased on those of the latter, with few exceptions. The exceptionally high counts on Marlboro in the Lloyd George-Marlboro pair and on Newman in the Lloyd George-Newman pair suggests that the aphids moved from the Lloyd George to Marlboro and Newman.

TABLE 1.—*Population of Amphorophora rubi on paired canes of red raspberry varieties* [1] *held in Erlenmeyer flasks in the greenhouse for 7 days*

Variety	Antwerp		Chief		Cuthbert		Herbert		King		Latham		Lloyd George		Marlboro		Newburgh		Newman	
Chief	87	1	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
Cuthbert	89	2	58	38	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
Herbert	7	4	15	34	1	[2] 20	----	----	----	----	----	----	----	----	----	----	----	----	----	----
King	47	5	39	20	73	43	51	9	----	----	----	----	----	----	----	----	----	----	----	----
Latham	60	0	61	61	24	[2] 27	11	7	15	89	----	----	----	----	----	----	----	----	----	----
Lloyd George	0	3	3	46	1	57	0	5	2	54	3	31	----	----	----	----	----	----	----	----
Marlboro	86	7	68	86	55	21	75	12	29	29	39	5	101	0	----	----	----	----	----	----
Newburgh	13	0	23	41	2	57	15	20	28	78	13	25	1	1	9	42	----	----	----	----
Newman	97	4	36	99	27	42	28	7	76	83	21	74	123	0	27	140	21	6	----	----
Viking	47	0	54	41	19	31	28	7	24	22	107	15	61	0	96	33	63	13	61	14

[1] The figures in the column at the left under each variety give the number of aphids on the variety listed in the stub; the figures in column at the right give the number of aphids on the variety at the top of the column.

[2] Larvae of syrphid fly present on the Cuthbert cane only.

The few aphids that remained on the Lloyd George canes were adults, indicating that reproduction had not occurred. The populations on Antwerp were small, but the presence of first instars indicated that reproduction had taken place. The number of instars found on Herbert and Newburgh showed that reproduction had taken place to a slightly greater extent than on Antwerp.

The behavior of *Amphorophora rubi* when confined to Antwerp, Herbert, Lloyd George, and Newburgh indicated that these varieties possess a marked degree of resistance to this mosaic vector.

In the second experiment, young succulent potted plants of the same varieties used in the first experiment were placed in the open insectary under voile cloth cages, each containing a single aphid-free plant. Twelve adult aphids were placed on each plant. The aphid counts on the different varieties after 7 days were as follows: Antwerp, 5; Chief, 77; Cuthbert, 74; Herbert, 42; King, 86; Latham, 88; Lloyd George, 0; Marlboro, 69; Newburgh, 23; Newman, 109; and Viking, 73. These data also indicated a marked resistance in Antwerp and Lloyd George, and a less marked resistance in Herbert and Newburgh, in contrast to the susceptibility of the other varieties.

During the growing season of 1936, an experiment was conducted in which a young vigorous plant of each of 15 varieties was placed in a cage by itself, and 10 aphids, second and third instars, were distributed on the tip leaves of each plant. Counts of the aphid populations were made 16 days later. The results showed more than 200 aphids (adults and instars) on each of Cayuga, Chief, Cuthbert, June, King, Latham, Marlboro, Newman, Preussen, Seneca, and Viking. No aphids were found on Herbert, Lloyd George, or Newburgh, and the population on Antwerp was small. The experiment was repeated by placing 25 aphids, second and third instars, on a caged plant of each of these four varieties. Counts of aphids made 14 days later were as follows: Antwerp, 32; Herbert, 6; Lloyd George, 0; and Newburgh, 21.

Since no aphids were found on the Lloyd George in the final count in each of the preceding experiments, a third test was conducted on this variety. Aphids were confined by means of ventilated cellophane bags to the tips of succulent canes on six potted Lloyd George plants. A leaf harboring numerous aphids of all stages was placed on the tip of the cane in each bag. When examined 16 days later, the aphids in all bags were dead.

During the growing season of 1937, the 15 varieties tested in 1936 were retested. The results confirmed those obtained in 1936. The experiment also included 10 additional varieties. *Amphorophora rubi* failed to maintain its population on three of these, namely, Indian Summer, Pyne Imperial, and Pyne Royal. These three varieties were tested a second time, and again the aphids failed to maintain their populations. Aphids fed and reproduction took place on the other seven varieties: Katherine, Laxton Bountiful, Laxton Renown, Marcy, Red Cross, Rote Riesen, and Taylor. Marcy showed some resistance in that the aphid population was low in comparison with that on the other varieties.

Table 2 summarizes the results of experiments conducted in the greenhouse during 1936 and 1937 for aphid resistance in red raspberry varieties based on their relative susceptibility.

TABLE 2.—*The reaction of 25 varieties of red raspberry to Amphorophora rubi under greenhouse conditions during 1936 and 1937*

Variety	Susceptible [1]	Partly resistant [2]	Resistant [3]	Variety	Susceptible [1]	Partly resistant [2]	Resistant [3]
Antwerp		(*)		Marcy		(*)	
Cayuga	(*)			Marlboro	(*)		
Chief	(*)			Newburgh		(*)	
Cuthbert	(*)			Newman	(*)		
Herbert		(*)		Preussen	(*)		
Indian Summer			(*)	Pyne Imperial			(*)
June	(*)			Pyne Royal			(*)
Katherine	(*)			Red Cross	(*)		
King	(*)			Rote Riesen	(*)		
Latham	(*)			Seneca	(*)		
Laxton Bountiful	(*)			Taylor	(*)		
Laxton Renown	(*)			Viking	(*)		
Lloyd George			(*)				

[1] The asterisk (*) indicates that the number of aphids was greater than 20 times the original number placed on the plant.
[2] The asterisk (*) indicates that the number of aphids was less than 5 times the original number placed on the plant.
[3] Aphids failed to reproduce and maintain a population.

FIELD OBSERVATIONS

Aphid counts were made in the field plots, July 2, 1935, on the 11 red raspberry varieties used in the first greenhouse experiment. The average number of aphids per cane was as follows: Antwerp, 11.5; Chief, 129.9; Cuthbert, 57.6; Herbert, 11; King, 66.5; Latham, 90.7; Lloyd George, 0; Marlboro, 69.6; Newburgh, 13.1, Newman, 86.3; and Viking, 87.

Aphid counts were made on the same varieties on several dates in 1936, as shown in table 3.

TABLE 3.—*Range [1] and average number of Amphorophora rubi on varieties of red raspberry in experimental plots, 1936*

Variety	May 25		June 9		June 25		July 7		July 25		Aug. 12		Aug. 28	
	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average
Antwerp	0-2	0.8	1-8	0.8	0-2	0.2	0-1	0.1	0-5	1.8	0-26	4.9	0-8	2.0
Chief	2-42	19.4	9-43	21.7	8-104	42.7	13-82	39.4	[2]3-108	41.1	16-64	44.9	6-55	22.4
Cuthbert	0-2	.5	0-7	2.6	0-16	5.0	0-11	5.0	[2]0-30	10.8	1-61	16.0	0-29	10.0
Herbert					0-3	.3	0-10	1.4	[2]0-6	1.8	0-6	2.4	0-6	.9
King					9-46	23.3	0-32	9.4	0-29	10.9	3-36	19.5	0-7	2.7
Latham	0-28	13.4	1-33	9.1	2-27	14.7	6-28	16.4	[2]4-49	23.1	3-54	21.9	0-26	6.2
Lloyd George	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Marlboro	0-8	3.7	0-18	5.1	1-9	4.6	4-21	7.7	4-42	16.8	1-13	6.1	0-4	1.7
Newburgh					0-6	2.0	0-13	4.7	[2]0-9	4.6	1-8	5.1	0-6	1.8
Newman	3-13	6.1	3-60	16.6	4-17	10.1	2-48	14.7	1-26	12.5	3-26	10.9	0-21	5.9
Viking	1-29	8.9	0-20	6.8	0-39	16.0	2-24	12.7	3-83	32.8	10-89	33.7	1-63	15.8

[1] The figures under range indicate the lowest and the highest aphid counts on individual canes.
[2] Count made July 23.

No aphids were found feeding on the Lloyd George at any time during the season.[6] Populations were generally low on Antwerp, Herbert, and Newburgh. The highest population was found on Chief. In general, the aphid population was greatest during the latter part of July and the fore part of August. According to the counts, aphids were not as numerous at any time during 1936 as in 1935.

[6] An occasional winged individual was found on the Lloyd George, which probably landed there accidentally while in flight.

Aphid counts [7] were made in commercial plantings of 36 different growers in the Puyallup Valley during July and early in August 1936. On the Cuthbert variety there was a great variation in aphid populations in different plantings even though the counts were made on the same date. For instance, counts made July 30 in one field showed an average of 36.7 aphids per cane, while in another field, located within a distance of one-half mile, there was an average of 4.1 aphids per cane. On Marlboro the aphid populations were small in the 12 plantings examined. An average of 4.4 aphids per cane was the heaviest infestation observed. Two Antwerp plantings were examined. One showed an average of two aphids per cane on July 8 and the other, examined July 28, showed none. On Lloyd George no aphids were observed in the six plantings examined.

During the growing season of 1937, a systematic aphid count was made in the experimental plots on 25 varieties. They were examined at intervals of 2 weeks or oftener, beginning May 14 and continuing until August 17. The results of the counts are summarized in table 4.

TABLE 4.—*Relative resistance of red raspberry varieties to Amphorophora rubi in experimental plots during 1937*

Variety	Times examined	Susceptible [1]	Partly resistant [2]	Resistant [3]
Antwerp	23		(*)	
Cayuga	12	(*)		
Cuthbert	21	(*)		
Herbert	23		(*)	
Indian Summer	10			(*)
June	5	(*)		
Katherine	5	(*)		
King	10	(*)		
Latham	17	(*)		
Laxton Bountiful	5	(*)		
Laxton Renown	5		(*)	
Laxton Reward	5	(*)		
Lloyd George	26			(*)
Marcy	11		(*)	
Marlboro	13	(*)		
Newburgh	29	(*)		
Newman	7	(*)		
Preussen	5	(*)		
Pyne Imperial	5			(*)
Pyne Royal	5			(*)
(St. Regis) Ranere	12	(*)		
Red Cross	5		(*)	
Rote Riesen	10	(*)		
Seneca	12	(*)		
Taylor	10	(*)		

[1] The asterisk (*) indicates that the average number of aphids was more than 5 per cane on any 1 date.
[2] The asterisk (*) indicates that the average number of aphids was less than 5 per cane on any 1 date.
[3] Aphids absent.

In the field counts on the 25 varieties shown in table 4, *Amphorophora rubi* was not found on 4 and only a few aphids were found on 5 varieties. Seldom were more than three aphids found on any one cane of Antwerp at one time. Observations made in commercial plantings and experimental plots during 1935, 1936, and 1937 revealed that spread of mosaic was very slow in this variety, even in plantings where it was interplanted in the same row with mosaic Cuthbert plants. One count on Herbert, made August 2, showed an average of 5.5 aphids per cane in one of the plots. With few exceptions, the aphid populations on this variety were small. At times, Newburgh was found quite susceptible [8] to aphid infestation in the field even though greenhouse experiments and some field examinations indicated

[7] Under a cooperative agreement with the State Department of Agriculture aphid counts were made by one of its men while he was inspecting commercial fields for mosaic.
[8] On one examination, August 10, an average of 34.6 aphids per cane were counted on Newburgh.

it to be partly resistant. Newburgh is classified in table 4 as susceptible in order to comply with the method of classification used.

RESISTANCE IN HYBRIDS

GREENHOUSE EXPERIMENTS

In 1936, greenhouse experiments with hybrids were limited to 50 selections from various crosses, including the resistant Lloyd George and several susceptible varieties. The results indicated that 6 of the hybrids were resistant to *Amphorophora rubi*, 13 partly resistant, and 31 susceptible. Of the hybrids in which resistance was indicated, one parent was the Lloyd George variety. A few hybrids between two susceptible parents were all susceptible.

In 1937, the 50 hybrids previously tested were retested, and in addition, all available inbred (self-pollinated) seedlings of Chief, Cuthbert, Latham, Lloyd George, and Newman were tested to obtain information as to the genetic constitution of these varieties in relation to aphid resistance. Fifty hybrids selected at random from crosses of Lloyd George and Chief also were tested. Crosses between these varieties were selected because of the marked aphid resistance in the Lloyd George and the marked aphid susceptibility in the Chief.

A summary of the results of the greenhouse experiments obtained in 1936 and 1937 is presented in table 5. Aphid populations on the susceptible seedlings ranged from 36 to more than 200 per cane tip. In a few cases, the aphid counts on plants of this group were less than 100, but in the majority of cases the counts exceeded 200. The relatively low counts on the partly resistant group contrasted sharply with the high aphid counts on the susceptible group. As the data of table 5 indicate, all inbred seedlings of the susceptible varieties, Chief, Cuthbert, Latham, and Newman showed definite susceptibility to *Amphorophora rubi*. Sixteen inbred seedlings of the resistant Lloyd George exhibited marked differences in susceptibility, only four being completely resistant and two partly resistant. The results of the tests of hybrids show definitely that Lloyd George transmits resistance in hybridization with susceptible varieties.

TABLE 5.—*Summary of aphid-resistance studies with red raspberry seedlings in the greenhouse during 1936 and 1937*

Seedlings tested (number)	Parentage	Susceptible [1]	Partly resistant [2]	Resistant [3]
5	Chief, self-pollinated	5		
6	Cuthbert, self-pollinated	6		
6	Latham, self-pollinated	6		
16	Lloyd George, self-pollinated	10	2	4
17	Newman, self-pollinated	17		
17	Lloyd George, open-pollinated	7	2	8
25	Chief × Lloyd George	16	1	8
25	Lloyd George × Chief	11		14
12	Cuthbert × Lloyd George	9	3	
16	Lloyd George × Cuthbert	9	4	3
4	Latham × Lloyd George	2	1	1
4	Lloyd George × Latham	3		1
1	King × Lloyd George		1	
1	Lloyd George × King		1	
2	Antwerp × Lloyd George	2		
2	Cuthbert × Latham	2		
2	Latham × Cuthbert	2		
1	Cuthbert × Marlboro	1		
1	Marlboro × Cuthbert	1		

[1] Average number of aphids more than 10 per cane tip.
[2] Average number of aphids less than 10 per cane tip.
[3] Aphids all dead.

FIELD OBSERVATIONS

Aphid counts were made in experimental plots only on those hybrids that had been selected for propagation because of superior fruit quality. All of these hybrids were included in the greenhouse investigations. The results of the field observations were in close agreement with those of the greenhouse experiments. Hybrids that harbored numerous aphids in the field showed susceptibility to aphids in the greenhouse and those that showed complete resistance in the greenhouse were consistently free of aphids in the field plots. Hybrids showing partial resistance in the greenhouse harbored few or no aphids in the field.

DISCUSSION

This investigation was undertaken to determine the practicability of attacking the red raspberry mosaic problem in western Washington by plant-breeding methods. In combating the mosaic disease, a variety that resists attack by mosaic vectors, and thereby escapes infection, will serve the same purpose as one that is resistant to the mosaic virus. The occurrence of resistance to *Amphorophora rubi* in important varieties such as Lloyd George therefore enhances their horticultural value. The fact that resistance is transmitted in hybridization offers a practical means of solving the mosaic problem in districts where *A. rubi* is the principal mosaic vector.

Throughout the investigation, there was a consistent agreement between the results of field observations and the results of greenhouse experiments. For example, the data in table 3, for field conditions, indicate the same relative susceptibility of varieties as the data of table 1, for greenhouse experiments.

It would be difficult to obtain an accurate comparison of varieties relative to aphid resistance by field observations alone, because of differences in aphid populations upon the same variety and rapid fluctuations caused by weather conditions. In comparison, greenhouse testing is more accurate because the aphids maintain steady populations and have an opportunity for maximum reproduction. Small aphid populations under greenhouse conditions should, therefore, be more significant than small populations under field conditions. On the other hand, greenhouse conditions may magnify the susceptibility of some varieties and hybrids that are relatively resistant under field conditions. The observation of the host-vector relationship in the field, therefore, is important as a supplement to greenhouse experiments.

The classification of varieties and hybrids into susceptible, partly resistant, and resistant groups is convenient for purposes of comparison but may not fully express the relative resistance of varieties except for those that are completely resistant. In the partly resistant group, Newburgh occasionally harbored larger numbers of *Amphorophora rubi* (footnote 8) than Herbert and Antwerp under similar conditions. Likewise, in the susceptible group, some varieties appeared to be more satisfactory than others as hosts for *A. rubi*. This suggests that a more detailed study of susceptibility might either indicate a more accurate grouping or eliminate grouping and show a gradation from complete resistance to high susceptibility. The experiments with hybrids do not clarify the situation. Aphid counts on 25 hybrids of

the cross Lloyd George × Chief (table 5) indicated only two classes. In other crosses, a wider variation in aphid populations was observed.

The results obtained in testing varieties and seedlings show that a variety may be completely resistant to *Amphorophora rubi* without being homozygous for resistance. Lloyd George is heterozygous because it transmits resistance to only a portion of its inbred and hybrid seedlings.

Although the number of seedlings tested was relatively small, it appears reasonably certain that Chief, Cuthbert, Latham, and Newman are homozygous for susceptibility. It remains to be determined whether any existing varieties are homozygous for resistance and whether homozygous resistant hybrids can be produced by hybridization.

SUMMARY

An investigation was undertaken during 1935, 1936, and 1937, to determine the resistance of named varieties and hybrids of red raspberry to the mosaic vector *Amphorophora rubi* Kalt. The study revealed that varieties of red raspberry possess various degrees of resistance to *A. rubi* in western Washington.

In the greenhouse *A. rubi* failed to reproduce and maintain its population on the varieties Indian Summer, Lloyd George, Pyne Imperial, and Pyne Royal. Reproduction was slow and the population remained small on the varieties Antwerp, Herbert, Marcy, and Newburgh. Aphids fed and reproduced abundantly on 19 varieties; namely, Cayuga, Chief, Cuthbert, June, Katherine, King, Latham, Laxton Bountiful, Laxton Renown, Laxton Reward, Marlboro, Newman, Preussen, Ranere (St. Regis), Red Cross, Rote Riesen, Seneca, Taylor, and Viking.

In experimental plots and commercial plantings *A. rubi* was found not to feed on those varieties that showed resistance to the aphid in the greenhouse. Populations were small on those showing partial resistance and comparatively large on the susceptible varieties.

Greenhouse experiments and field observations with red raspberry seedlings showed resistance to *A. rubi* to be inherited and transmitted when a resistant variety was crossed with a susceptible one. The variety Lloyd George was found to be heterozygous for resistance.

Greenhouse testing is a convenient and rapid method for determining the relative resistance of varieties and hybrids of red raspberry to *A. rubi*.

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